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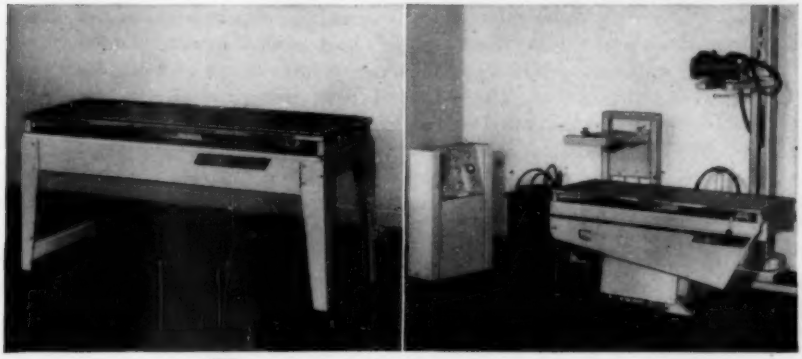

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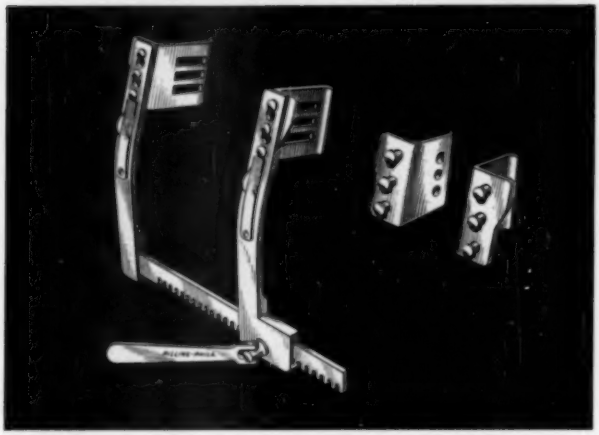

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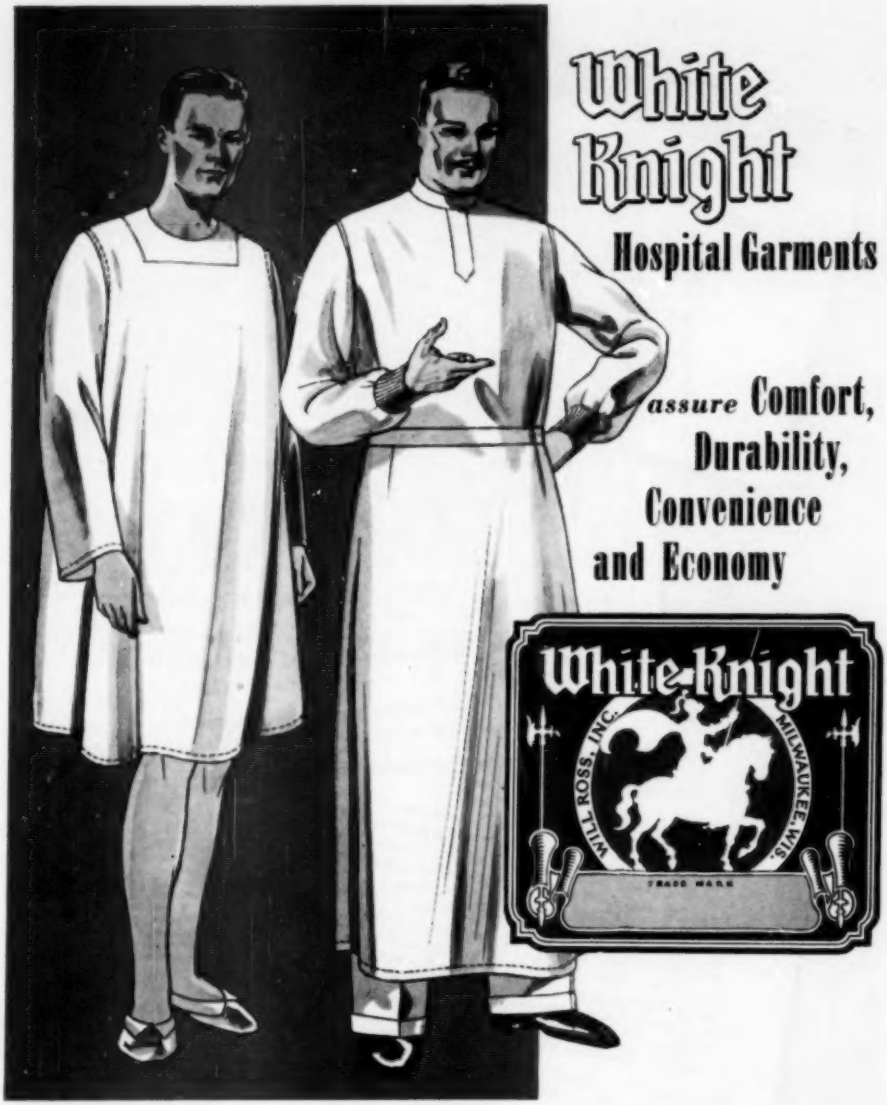

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VIOMYCIN A NEW ANTIBIOTIC ACTIVE AGAINST MYCOBACTERIA[1]

A. C. FINLAY, G. L. HOBBY, F. HOCHSTEIN, T. M. LEES, T. F. LENERT, J. A. MEANS, S. Y. P'AN, P. P. REGNA, J. B. ROUTIEN, B. A. SOBIN, K. B. TATE, AND J. H. KANE

(Received for publication August 23, 1950)

INTRODUCTION

Viomycin[2] is a new antimicrobial agent (1, 2, 3) produced by an actinomycete which has been named *Streptomyces puniceus* because of the purplish-red color of its vegetative mycelium. The antibiotic-producing property of this organism was first demonstrated in the course of a large-scale screening program in which the actinomycete was grown in the form of a single colony in the center of Petri dishes containing nutrient agar. When the diameter of the colony had reached approximately 1 cm., streaks of various bacteria were made away from the colony. The test strains included a variety of gram-negative enteric bacteria, aerobic spore-formers, gram-positive cocci, and *Myocobacteria*. After further incubation, it was observed that growth of many of the test organisms was inhibited in the vicinity of the colony. The strain of *Streptomyces puniceus* grew readily under submerged aerobic conditions and the broth exhibited similar inhibitory power, as demonstrated in serial dilution assays. From broth cultures of this organism an antibiotic was isolated and the generic name, viomycin, assigned to it.

Viomycin differs from many other antibiotics in that it is relatively more active against the *Mycobacteria* than against other species. When various acid-fast bacteria, including *M. ranae*, *M. phlei*, *M. smegmatis*, and *M. tuberculosis* ATCC 607 are inoculated onto plates of Youmans' synthetic agar medium containing various concentrations of viomycin, growth is inhibited by approximately 10 γ[3] of the antibiotic per ml. of medium. In contrast, considerably larger amounts

[1] From the Research Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, New York.

[2] Viomycin was discovered and developed independently by Chas. Pfizer & Co., Inc., and by Parke, Davis & Company. The organisms producing this antibiotic have been designated *Streptomyces puniceus* by the investigators at Chas. Pfizer & Co., and *Streptomyces floridae* by those at Parke, Davis & Company. No comparison has been made as yet to determine the possible identity of these two strains.

[3] The unit of activity of the preparations was defined as 1 γ of the pure base and the activities of the salts are stated throughout in terms of the equivalent weight of pure viomycin base. All assays were performed with a modified turbidimetric assay procedure (4), using *K. pneumoniae* P.C.I. 602 as the test organism.

of the antibiotic are necessary to prevent growth of nonacid-fast bacteria when tested in the same manner.

PROPERTIES OF VIOMYCIN

Viomycin is a strong organic base and forms essentially neutral salts. The sulfate and oxalate have been prepared in crystalline form. Hydrated viomycin sulfate has the following properties: It decomposes at 280°C. and its $[\alpha]_D^{25} = -32°$ (c, 1 per cent in water). It is highly soluble in water and virtually insoluble in most organic solvents. Aqueous solutions of viomycin sulfate are moderately stable at pH 5–6 at room temperature. At 100°C., the half life of such solutions is approximately 12 hours. A sample of crystalline anhydrous

TABLE 1

In Vitro Activity of Viomycin on M. Tuberculosis

STRAINS OF M. TUBERCULOSIS	MINIMAL INHIBITORY CONCENTRATION,* γ PER ML.	
	Viomycin	Streptomycin
H37Rv (#2290)	2.8	0.25
(#1337)	1.0	2,500.00
Freshly isolated strains	1.9–2.8	0.25–25.0
	1.9–5.6	>1,000–>5,000

* Readings after 12 days' incubation in liquid Tween-albumin medium.

TABLE 2

*Chemotherapeutic Action of Viomycin in Experimental Tuberculous Infections in Mice**

DAILY DOSE	AVERAGE SURVIVAL TIME	PERCENTAGE SURVIVAL
mg. per kg.		
175	164	90
0	83	0

* Animals infected intracerebrally with *M. tuberculosis* (H37Rv) and viomycin administered subcutaneously for 4 weeks. Results calculated at 180 days after infection.

viomycin sulfate has given the following analysis: C, 37.19; H, 5.86; N, 20.61; SO_4, 17.63.

Acidic and basic hydrolysates of viomycin are biologically inactive. Intact viomycin contains about 2.7 per cent Van Slyke nitrogen, and titration curves show the presence of strongly basic groups. The antibiotic shows positive Sakaguchi and Fehling's tests.

TOXICITY

Viomycin is an antibiotic of moderately low toxicity. In mice, the intravenous LD_0 of viomycin sulfate is equivalent to 176 mg. of the free base per kg. of body weight, and the LD_{50} is equivalent to 240 mg. per kg. of body weight. No evidence of renal damage has been observed in rats receiving daily doses of 50

to 200 mg. of viomycin hydrochloride or sulfate per kg. of body weight subcutaneously for periods of five days. Moreover, daily dosages of 50 to 100 mg. per kg. of body weight, administered intramuscularly to dogs over prolonged periods of time, likewise have produced no signs of toxicity. Renal damage has been demonstrated in rats given daily doses of 400 mg. per kg. of body weight for five days, while ataxia and incoordination of gait have occurred frequently in cats receiving 100 or more mg. per kg. of body weight.

ACTIVITY ON TUBERCLE BACILLI IN VITRO AND IN VIVO

In table 1 may be seen the effect of viomycin *in vitro* on the growth of *M. tuberculosis*, including both streptomycin-sensitive and streptomycin-resistant strains. The high *in vitro* activity of viomycin against acid-fast bacteria and the low order of activity against the nonacid-fast bacteria suggested that this antibiotic might be capable of exerting a specific suppressive action on the course of tuberculous infection in animals and man. In experiments with mice infected with *M. tuberculosis* (H37Rv), daily doses of 175 mg. of viomycin sulfate per kg. of body weight protected 90 per cent of the animals thus treated; whereas none of the similarly infected but untreated animals survived (table 2).

It is known that the daily dose of other antibiotics (e.g., penicillin and streptomycin) required to protect mice against experimental infection with susceptible organisms is three or more times greater, as calculated on the basis of body weight, than that required to protect against similar infections in higher species. If this relationship proves equally true with viomycin, it is believed that a safe margin may exist between the protective and the toxic dose. Furthermore, preliminary observations in man (5) indicate that viomycin may be safely administered to humans over prolonged periods of time.

Viomycin offers promise as a chemotherapeutic agent in tuberculosis when used alone or in combination with other antimicrobial agents, and warrants further evaluation.

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VIOMYCIN, A NEW TUBERCULOSTATIC ANTIBIOTIC[1,2]

QUENTIN R. BARTZ, JOHN EHRLICH, JAMES D. MOLD, MILDRED A. PENNER, AND ROBERT M. SMITH

(Received for publication August 15, 1950)

Several isolates of a raspberry to violet-colored actinomycete have been obtained from soil samples and investigated because of their antagonism for *Mycobacteria*. The isolates represent an apparently new species of *Streptomyces* for which the name *Streptomyces floridae* has been proposed because the culture first studied intensively (A5014) was isolated from a Florida soil sample. In cross-streak tests on agar plates this isolate markedly inhibited the growth of *Mycobacterium tuberculosis* ATCC 607, *M. tuberculosis* var. *hominis* H37Rv, and the streptomycin-resistant H37RvR, and exhibited lesser activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus pyogenes* var. *aureus*, *Shigella sonnei*, *Candida albicans*, and *Trichophyton gypseum*. When the organism was grown in liquid media in shaken flasks, filtrates of these aerobic cultures exhibited *in vitro* activity against the bacteria listed and also against *Bacillus subtilis*, *Brucella suis*, *Salmonella schottmuelleri*, *Streptococcus viridans*, and *Trichomonas foetus*. The filtrates exhibited no activity against the fungi and viruses tested. An antibiotic was isolated from submerged aerated cultures of this organism.[3] The name viomycin is being assigned to this antibiotic.

The antibiotic was isolated and purified as the sulfate by adsorption on and elution from carbon (1) followed by repeated precipitation from 50 per cent aqueous methanol. The pure micro-crystalline sulfate, dried *in vacuo* at room temperature, melted at 252°C. with decomposition and had a specific rotation of $-39.8°$ (c = 1.0 in water at pH 6.0). It was noted that the latter varied somewhat with the pH of the solution. When a sample was dried at 100°C. for 16 hours over P_2O_5 (weight loss 10.7 per cent), the specific rotation, in aqueous phosphate buffer at pH 7.0, dropped from $-36.8°$ to $-19.6°$ without significant loss of biological activity; dried at 50°C. for 24 hours, the weight loss was 9.45 per cent and the rotation rose to $-40.6°$. Both the sulfate and the hydrochloride are very soluble in water and virtually insoluble in organic solvents, although the hydrochloride may be recrystallized from a large volume of absolute methanol. Other crystalline salts which have been prepared are the picrate and the

[1] From the Research Laboratories of Parke, Davis & Company, Detroit, Michigan.

[2] The authors acknowledge the participation in these studies of numerous individuals, particularly Paul R. Burkholder of Yale University, in whose laboratories the cultures were isolated and screened for antimicrobial activity; Guy P. Youmans of Northwestern University, who first demonstrated activity of the cultures and of our viomycin preparations against virulent human strains of tubercle bacilli *in vitro* and *in vivo*; and Lucia E. Anderson, A. C. Bratton, Jr., O. M. Gruhzit, A. S. Schlingman, John M. Vandenbelt, and their associates, all of Parke, Davis and Company, who have collaborated in various ways.

[3] Recently a sample of an antibiotic, discussed at the Ninth Veterans Administration Streptomycin Conference, and subsequently supplied to us by workers in the Research Laboratories of Chas. Pfizer & Company, Inc., was examined and found to be identical with our material.

reineckate. The antibiotic is very stable in acid solutions, less so in base. Tests of culture filtrate under various conditions of temperature and pH yielded the following data, expressed as percentage of the initial activity: pH 2, 25°C., 24 hours, 100 per cent; pH 2, 100°C., ½ hour, 100 per cent; pH 7, 25°C., 24 hours 100 per cent; pH 7, 100°C., ½ hour, 59 per cent; pH 9.5, 25°C., 24 hours, 81 per cent; pH 9.5, 100°C., ½ hour, 32 per cent.

TABLE 1

Antibacterial Activity of Viomycin Sulfate in Vitro

SPECIES	BACTERIOSTATIC CONCENTRATION
	γ per ml.
Mycobacterium tuberculosis ATCC 607*	0.78
Mycobacterium ATCC 607, streptomycin-resistant*	0.78
Mycobacterium ATCC 607, neomycin-resistant*	0.78
M. tuberculosis var. *hominis* H37Rv*	6.25–12.5
M. tuberculosis var. *hominis* H37Rv, streptomycin-resistant*	6.25–12.5
M. tuberculosis var. *hominis*, 16 clinical isolates	3.12– 6.25
M. tuberculosis var. *hominis*, 16 clinical isolates*	6.25–12.5
Klebsiella pneumoniae, type A	10.0
Salmonella typhosa	10.0 –25.0
Escherichia coli, streptomycin-resistant	10.0
Escherichia coli, parent culture	25.0
Neisseria catarrhalis	10.0 –25.0
Neisseria meningitidis	25.0
Aerobacter aerogenes	25.0
Corynebacterium diphtheriae	10.0 –25.0
Diplococcus pneumoniae	50.0
Brucella abortus	50.0
Salmonella schottmuelleri	100
Shigella paradysenteriae	100
Brucella suis	>100
Micrococcus pyogenes var. *aureus*	>100
Pasteurella bollingeri	>100
Proteus sp.	>100
Pseudomonas aeruginosa	>100
Streptococcus hemolyticus	>100
Vibrio comma	>100

* Serum present in culture medium.

Qualitative tests pertinent to the component groups of the compound were, in part: positive Sakaguchi (guanidine present), positive ninhydrin, negative maltol and Benedict's, and positive biuret test (peptide linkage present). The ultraviolet absorption spectrum of the sulfate shows a strong maximum which shifts with change of pH: $E^{1\%}_{1cm}$ 339 at 268 mμ (0.1N HCl), 334 at 268.5 mu (pH 7), 219 at 282.5 mu (0.1N NaOH). The infra-red spectrum (Nujol mull against Nujol blank) shows bands at 3.06, 3.40[4], 5.97, 6.06, 6.16 (s)[5], 6.43, 6.51,

[4] Nujol mull contributing.

[5] (s) Shoulder on a stronger band. Allow ±0.02 μ for error of calibration and measurement.

6.60, 6.65, 6.71 (s), 6.87[4], 7.30[4], 7.49, 7.61 (s), 8.15, 8.65 (s), 9.23, 13.85 μ. Elementary analysis of a dried sample of the antibiotic sulfate gave C, 35.83; H, 5.77; N, 21.08; S, 5.34; Van Slyke amino nitrogen, 2.5, 2.6 at 4 minutes, 4.51 at 2 hours; no halogen or phosphorus.

The antibiotic proved to be moderately active *in vitro* against certain bacteria, notably tubercle bacilli, and relatively inactive against other bacteria, as well as the yeasts and filamentous fungi, protozoa, and viruses tested. *In vitro* activity against various bacteria is exemplified by the data in table 1. Varying amounts of the sulfate salt were dissolved in approximately neutral broth media which were then inoculated with the test organisms and incubated at 37°C.

The highly purified antibiotic is well tolerated by laboratory animals. Single intravenous injections of 250 mg. of the hydrated sulfate per kg. and subcutaneous injections of 1,000 mg. per kg. caused no fatalities in mice. Repeated subcutaneous injections of 300 mg. per kg. twice daily for 2 weeks were well tolerated by mice.

The antibiotic is active on tubercle bacilli *in vivo* as well as *in vitro*, since it exhibits a marked protective action against experimental tuberculosis in mice.

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ANTIMICROBIAL ACTIVITY OF STREPTOMYCES FLORIDAE AND OF VIOMYCIN[1]

JOHN EHRLICH, ROBERT M. SMITH, MILDRED A. PENNER, LUCIA E. ANDERSON, AND A. C. BRATTON, JR.

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INTRODUCTION

Among numerous cultures of actinomycetes isolated from soils which were being screened for antibiotic activity was a group that attracted early attention because of their predominant activity against *Mycobacteria*. These cultures are believed to represent a new species designated by the name *Streptomyces floridae*. Culture filtrates from several of these isolates yielded an antibiotic substance which subsequently has been named viomycin (1). The purpose of this paper is to report data on the antimicrobial activity of the cultures and of viomycin.

ANTIMICROBIAL ACTIVITY OF STREPTOMYCES FLORIDAE

Agar cultures: The cultures were first screened for antibacterial and antifungal activity by the cross-streak method on several agar media in Petri dishes. In table 1 the results of such tests with three representative isolates are given. The noteworthy characteristic of these data is the marked activity of the cultures against *Mycobacteria* and their indifferent activity against most of the other bacteria and fungi tested, indicating the presence of at least one presumably new antibiotic potentially useful in the treatment of tuberculosis.

Broth cultures: The cultures were grown submerged in broth media and tests were made of the antibacterial activity of shaken culture filtrates. In table 2 are presented the results of these tests. These results showed that *Streptomyces floridae* was able to produce in submerged culture a distinctive pattern of antimicrobial activity similar to that exhibited on agar. The shaker filtrate pattern was characterized by substantial activity against *Mycobacterium tuberculosis* var. *hominis*, lesser activity against various gram-positive and gram-negative bacteria and *Trichomonas foetus*, and inactivity against *Endamoeba histolytica* and the fungi and viruses tested.

ANTIMICROBIAL ACTIVITY OF VIOMYCIN SULFATE

From submerged culture filtrates Barts and Mold (1) obtained a highly purified, neutral, water-soluble organic sulfate, which has been designated by the name viomycin sulfate. Biochemical investigation was guided by a microbiological disc-plate assay with *Bacillus subtilis* and *Escherichia coli*, activity being expressed in terms of equivalent streptomycin units. When the pure compound became available, an agar diffusion assay was devised, employing as test organism *Bacillus subtilis* or *Mycobacterium tuberculosis* ATCC 607. In this test, activity is expressed gravimetrically in terms of a viomycin sulfate standard.

[1] From the Research Laboratories of Parke, Davis & Company, Detroit, Michigan.

TABLE 1

Agar Cross-streak Data on Three Isolates of Streptomyces Floridae

TEST SPECIES	STRAIN	MEDIA*						
		1	2	3	4	7	8	9
		width of zone of inhibition (mm.)						
Escherichia coli	PD 01495	0–5.0	0–4.0	0	0	0	0	—
Escherichia coli‡	Waksman	—	—	—	—	—	—	0–3p†
Klebsiella pneumoniae, type A	PD 04544	0–12	0–10	0	0	0	0–10	—
Micrococcus pyogenes var. *aureus*‡	Cowles	—	—	—	—	—	—	13–>30
Micrococcus pyogenes var. *aureus*	ATCC 6538	5p–13	0–17	0–10	0–12	0–6.0	0–9.0	—
Mycobacterium tuberculosis 607‡	ATCC 607	—	—	—	—	—	—	23–>30
M. tuberculosis var. *hominis*§	NIH H37Rv	p–16.5	p–8.0	19	17	—	p	—
M. tuberculosis var. *hominis*§	Youmans H37RvR‖	p–18	p–8.0	14	10	—	p	—
Pseudomonas aeruginosa	PD 01925	0	0	0	0	0	0	—
Salmonella schottmuelleri	NIH Cools	0–5.0	0–6	0	0	0	0	—
Shigella sonnei	PD 04628	0–15	0–11	0	0	0–12	0–9.0	—
Streptococcus hemolyticus	PD 04472	0–6.0	0–8.0	0–4.0	0–4.0	0	0–3.0	—
Candida albicans‡	Clinical isolate	—	—	—	—	—	—	2.5–5.0
Candida albicans	PD 04600	0–4.0	0–1.0	0	0	0	0	—
Epidermophyton floccosum	PD 04624	0–9.0	0–11	0–6.0	0–8.0	0	0	—
Microsporum audouini	ATCC 10008	0–11	0–10	0–5.0	0	0	0	—
Microsporum canis	ATCC 10214	0–2.0	0	0	0	0	0	—
Trichophyton gypseum‡	Clinical isolate	—	—	—	—	—	—	0–2.0
Trichophyton mentagrophytes (*interdigitale* type)	ATCC 9533	0–3.0	0	0	0	0	0	—
Trichophyton rubrum	ATCC 9971	0–6.0	0–5.0	0	0–4.0	0	0	—

* For composition of media, see Smith *et al.* (1948), table 1, footnote‡, page 426.

†p = partial inhibition.

‡ Tested shortly after isolation by Dr. Paul R. Burkholder, Yale University.

§ Tested by Dr. Guy P. Youmans, Elizabeth H. Williston, and Pari Zia-Walrath, Northwestern University Medical School.

‖ H37RvR is Dr. Youmans' streptomycin-resistant substrain of NIH H37Rv.

Others tested by Dwight A. Joslyn and Betty J. Beck of Parke, Davis & Company.

In table 3 the results of tests of *in vitro* activity of viomycin sulfate on a number of bacteria and actinomycetes are shown. The antibiotic was also tested and found to be inactive at a concentration of 250 γ per ml. of broth against several

TABLE 2

*Antimicrobial Activity of Shake Culture Filtrates of Streptomyces Floridae**

TEST SPECIES	STRAIN	TEST METHOD	EQUIVALENT STREPTOMYCIN	GROWTH INHIBITION	RECIPROCAL OF MAXIMAL INHIBITORY DILUTION
			units/ml.	*per cent*	
Bacillus subtilis	ATCC 6633	Agar diffusion	2.9	—	—
Escherichia coli	ATCC 9637	Agar diffusion	6.3	—	—
M. tuberculosis var. *hominis*	NIH H37Rv	Broth dilution	—	100	32
M. tuberculosis var. *hominis*	NIH H37Rv	Broth dilution + 10% beef serum	—	100	16
M. tuberculosis var. *hominis*	Youmans H37RvR	Broth dilution	—	100	64
M. tuberculosis var. *hominis*	Youmans H37RvR	Broth dilution + 10% beef serum	—	100	32
Brucella suis	Huddleson 1772	Agar dilution	—	100	50
Escherichia coli	PD 01495	Turbidimetric	—	45	10
Klebsiella pneumoniae, type A	PD 04544	Turbidimetric	—	43	10
Micrococcus pyogenes var. *aureus*	ATCC 6538	Turbidimetric	—	82	50
Salmonella schottmuelleri	NIH Cools	Turbidimetric	—	62	10
Shigella sonnei	PD 04628	Turbidimetric	—	76	10
Streptococcus viridans	PD 04409	Turbidimetric	—	88	10

* Isolate No. A5104, No. 8 Broth. Seitzed filtrates from this or other media were also tested and found to be inactive against *Aerobacter aerogenes* PD 0126, *Hemophilus pertussis* PD 04692, *Pseudomonas aeruginosa* PD 01925, *Shigella paradysenteriae* PD 04651, *Candida albicans* PD 04600, *Microsporum audouini* ATCC 10008, *Trichophyton mentagrophytes* (*interdigitale* type) ATCC 9533, *Endamoeba histolytica* Univ. Chicago strain, *Borreliota variolae* City of N. Y. Board of Health strain, *Erro ecclestus* Hubbard strain, *Rabula inflans* Enders strain, *Tarpeia alpha* NIH strain, and *Tortor furens* California strain from BAI. A filtrate was lethal for *Trichomonas foetus* B. Morgan strain. *Mycobacteria* were tested by G. P. Youmans. Other bacteria were tested by M. E. Doles, B. M. Duckworth, M. Galbraith, F. E. Guest, M. J. Hemans, D. L. Kohberger, M. C. Manning, R. W. Sarber, A. S. Schlingman, and M. F. Sheehan; fungi, by A. B. Hillegas and B. D. Mercer; protozoa, by A. Bayles, B. L. Lilligren, T. F. Reutner, and P. E. Thompson; viruses, by I. W. McLean, Jr., F. A. Miller, and A. Pucheu, Parke, Davis & Company.

eumycetes, including *Aspergillus fumigatus* NIH 5215, *Blastomyces dermatitidis* Conant 1035, *Candida albicans* PD 04600, *Cryptococcus neoformans* ATCC 10226, *Histoplasma capsulatum* Conant 1076, *Sporotrichum schenckii* ATCC

TABLE 3

Antimicrobial Activity of Viomycin Sulfate in Vitro

TEST SPECIES	STRAIN	CONCENTRATION CAUSING COMPLETE INHIBITION OF GROWTH				CONCENTRATION CAUSING 50 PER CENT INHIBITION OF GROWTH (TURBIDIMETRICALLY)		
		pH 7.1	pH 7.8	pH 8.4	Test Method	pH 7.2	pH 8.0	Test Method
		γ/ml.				γ/ml.		
Actinomyces bovis	PD 04	25	—	—	6	—	—	—
Aerobacter aerogenes	PD 0126	25	10	2.5	1	21.5	12.5	2
Bacillus subtilis	ATCC 6633	25	—	—	1	—	—	—
Brucella abortus	ATCC 7705	50	50	25	1	—	—	—
Brucella suis	Huddleson 1772	>100	100	50	1	—	—	—
Corynebacterium diphtheriae	PD 036	25	5	1	1	>40¶	>40¶	2
Diplococcus pneumoniae, type I	PD 04385	50	50	100	1	>40	>40¶	2
Escherichia coli	PD 01495	—	—	—	—	54	17.2	2
Escherichia coli var. *communior*	PD 04420	>100	50	50	1	55.8	25.7	2
Escherichia coli (parent)	Waksman 52	25	10	2.5	1	17.9	7.5	2
Escherichia coli (SM-R)	Waksman 52A*	10	5	1	1	33.3	8.3	2
Escherichia coli (ST-R)	PD 04862†	>100	>100	100	1	—	—	—
Hemophilus pertussis	PD 04692	50	—	—	3	—	—	—
Klebsiella pneumoniae, type A	PD 04544	10	5	1	1	39.5	14.9	2
Klebsiella pneumoniae	ATCC 10031	—	—	—	—	6.9	2.8	2
Micrococcus pyogenes var. *aureus*	ATCC 6538	>100	50	25	1	75.1->100	27.7-47.6	2
Mycobacterium tuberculosis	ATCC 607	0.78	—	—	4	—	—	—
Mycobacterium 607 (SM-R)	PD Bratton‡	0.78	—	—	4	—	—	—
Mycobacterium 607 (NM-R)	PD Bratton§	0.78	—	—	4	—	—	—
M. tuberculosis var. *hominis*	NIH H37Rv	2.5-7.8	—	—	5	—	—	—
M. tuberculosis var. *hominis* with 10% beef serum	NIH H37Rv	6.25-12.5	—	—	4a, 5	—	—	—
M. tuberculosis var. *hominis* (SM-R)	Youmans H37RvR‖	2.5-3.9	—	—	5	—	—	—
M. tuberculosis var. *hominis* (SM-R) with 10% beef serum	Youmans H37RvR‖	6.25-12.5	—	—	5	—	—	—

Neisseria catarrhalis	PD 03447	10	5	2.5	1	—	—	—
Neisseria meningitidis	NIH 1027	25	5	2.5	1	—	—	—
Nocardia asteroides	NIH 9966	25	—	—	6	—	—	—
Pasteurella bollingeri	PD 02855	>100	>100	>100	1	42.7¶	30.5	2
Proteus vulgaris	PD 04736	>100	>100	100	1	>100	>100	2
Pseudomonas aeruginosa	PD 01925	>100	>100	100	1	—	—	—
Salmonella schottmuelleri	NIH Cools	100	50	10	1	27	13.5	2
Salmonella typhosa	NIH Hopkins	10	5	2.5	1	13.7	6.6	2
Salmonella typhosa	PD 04815	—	—	—	—	100	27.7	2
Serratia marcescens	Sloan-Kettering 17	—	—	—	—	>100	55.8	2
Shigella paradysenteriae	PD 02904	100	50	10	1	33.5	17.9	2
Shigella sonnei	PD 04628	—	—	—	—	22.2	9.5	2
Streptococcus hemolyticus	PD 04472	>100	>100¶	—¶	1	—	—	—
Streptococcus hemolyticus	ATCC 9854	—	—	—	—	>100	>100	2
Streptococcus viridans	PD 04409	—	—	—	—	>100	>100	2
Vibrio comma, Inaba	NIH 35A3	>100	>100	>100	1	—	—	—

TEST METHOD	TEST MEDIUM	PROCEDURE	CONCENTRATIONS OF ANTIBIOTICS	TESTED BY
			γ/ml.	
1	Bacto-tryptose broth	Broth containing the antibiotic was inoculated with sufficient overnight broth culture, standardized in a "lumetron," to give minimal visible growth in the controls after 18 hours at 37°C.	100, 50, 25, 10, 5.0, 2.5, 1.0	A. S. Schlingman M. C. Manning M. E. Doles
2	Bacto-brain heart infusion broth	Broth containing the antibiotic was inoculated with sufficient 2-hour broth culture, standardized in a "lumetron," to give 35 per cent light transmittance in the controls after 3 to 5 hours at 37°C.	Endpoint read from curve for inhibition/concentration	M. Galbraith M. F. Sheehan M. A. Keilholz
3	Sauer human blood agar	Petri plates containing the antibiotic in the medium were surface-seeded with 10,000 phase-I organisms from 24-hour agar surface growth and incubated for 4 days at 36°C.	100, 50, 10, 7.5, 5.0, 1.0, 0.5	R. W. Sarber M. J. Hemans

TABLE 3—*Continued*

TEST METHOD	TEST MEDIUM	PROCEDURE	CONCENTRATIONS OF ANTIBIOTICS	TESTED BY
			γ/ml.	
4	Modified Proskauer-Beck broth containing 2% glycerin and 10% beef serum	Tubes containing the antibiotic in 4 ml. broth were inoculated with 0.05 mg. (moist weight) cells (prepared from a 5-day liquid culture by grinding with glass beads and standardizing by centrifugation in a Hopkins vaccine tube) and incubated for 5 days at 37°C.	Twofold dilutions starting at 100	A. C. Bratton, Jr. F. Montross
4a	Same as 4	Same as 4 except that inoculum and test cultures were incubated for 7 days	Same as 4	Same as 4
5	Youmans synthetic broth	Tubes containing the antibiotic in 5 to 10 ml. broth were inoculated with 0.1 to 0.5 mg. (wet weight) cells (prepared from a 21-day agar surface growth and standardized by centrifugation in a Hopkins vaccine tube) and incubated for 14 days at 37°C.	Twofold dilutions starting at 128 or 100	G. P. Youmans E. H. Williston P. Zia-Walrath A. S. Youmans
6	Bacto-brain heart infusion broth	Broth containing the antibiotic was inoculated with adequate number of mycelial fragments (prepared by grinding a week's agar surface growth, suspending in broth, filtering, and standardizing by hemocytometer) to give visible growth in the controls after 72 hours at 29°C.	100, 50, 25, 15, 10, 5.0, 2.5, 1.0	A. B. Hillegas B. D. Mercer

* Waksman's streptomycin-resistant substrain derived from Waksman 52 (= Murray 2).
† PD streptothricin-resistant substrain derived by Iverson from PD 01495.
‡ PD streptomycin-resistant substrain derived by Bratton from ATCC 607.
§ PD neomycin-resistant substrain derived by Bratton from ATCC 607.
‖ Youmans' streptomycin-resistant substrain derived from NIH H37Rv (= ATCC 9360).
¶ Growth of culture too light for valid test.

10213, and *Trichophyton mentagrophytes* (*interdigitale* type) ATCC 9533. It was inactive against two protozoans, 2 mg. per ml. of broth failing to inhibit *Endamoeba histolytica* (University of Chicago strain) and 5 mg. per ml. of saline solution killing *Trichomonas foetus* (B. Morgan strain) only after 25½ hours. Viomycin sulfate was also inactive against several small viruses, including *Erro scelestus* Hubbard strain (1 dose of 0.25 mg. per mouse or per egg), *Formido inexorabilis*

TABLE 4

Influence of Incubation Period on Antibacterial Activity of Viomycin Sulfate in Vitro[a]

TEST SPECIES	STRAIN	INITIAL REACTION OF MEDIUM	BACTERIOSTATIC CONCENTRATION AFTER INCUBATION FOR			
			24 hours	48 hours	72 hours	144 hours
		pH	γ/ml.			
Aerobacter aerogenes	PD 0126	7.1	25	50	50	50
		8.5	2.5	5	5	5
Brucella abortus	ATCC 7705	7.1	50	50	50	50
		8.5	10	10	10	10
Cornebacterium diphtheriae	PD 036	7.1	10	10	10	10
		8.5	1	1	1	1
Escherichia coli	Waksman 52	7.1	25	25	25	25
		8.5	2.5	5	5	5
Klebsiella pneumoniae	PD 04544	7.1	10	25	25	25
		8.5	1	1	2.5	2.5
Micrococcus pyogenes var. *aureus*	ATCC 6538	7.1	>100	>100	>100	>100
		8.5	25	50	100	100
Neisseria catarrhalis	PD 03447	7.1	25	25	25	25
		8.5	1	1	1	1
Salmonella typhosa	NIH Hopkins	7.1	25	>100	>100	>100
		8.5	5	25	25	25

[a] Test method No. 1: See table 3.

NIH CVS muscle-adapted strain (3 times 0.25 mg. per mouse), *Legio debilitans* Lansing mouse-adapted strain (3 times 0.25 mg. per mouse), *Rabula inflans* Enders strain (1 times 0.25 mg. per egg), *Tarpeia alpha* NIH strain (3 times 0.25 mg. per egg), and *Tortor furens* California strain (1 times 0.25 mg. per egg).[3]

The data in table 3 show that concentrations of 2.5 to 7.8 γ of viomycin sulfate per ml. of approximately neutral media completely inhibited growth, not only of the virulent human H37Rv strain of tubercle bacilli, but also of its streptomycin-

[3] See table 2, footnote.

resistant derivative. Moreover, its activity in the presence of 10 per cent serum was reduced by only approximately one-half. Viomycin in a concentration of 0.78 γ per ml. inhibited the avirulent *Mycobacterium tuberculosis* ATCC 607 and its streptomycin-resistant and neomycin-resistant derivatives. It is bacteriostatic at 10 or 25 γ per ml. against two actinomycetes and single strains of various gram-positive and gram-negative bacteria, including *Aerobacter aerogenes*, *Bacillus subtilis*, *Corynebacterium diphtheriae*, *Escherichia coli* and a streptomycin-resistant derivative, *Klebsiella pneumoniae*, *Neisseria catarrhalis*, *Neisseria meningitidis*, *Nocardia asteroides*, and *Salmonella typhosa*. The antibiotic is less active against the other bacteria tested.

It is apparent from the foregoing results that the antibiotic possesses little or no activity against *Micrococcus pyogenes* var. *aureus*, fungi, protozoa, and viruses. The activity exhibited by *Streptomyces floridae* on agar or in broth against certain of the fungi, *Trichomonas foetus*, and particularly *Micrococcus pyogenes* var. *aureus*, presumably reflects the presence in such cultures of nonspecific or specific antimicrobial material discarded in the course of fractionation of the culture liquid and isolation of viomycin.

The data in table 3 also show that the antibacterial activity of viomycin sulfate tended to be greater as the initial pH was increased within the range of pH 7.1 to 8.4. The extent of increase was variable, ranging from two- to twenty-five-fold.

To determine whether viomycin sulfate would maintain its bacteriostatic activity in broth culture, several of the test bacteria were incubated with the antibiotic in Bacto-tryptose broth at pH 7.1 and 8.5 for 6 days and examined at intervals for bacteriostatic levels. The data in table 4 show that with one exception the inhibitory concentration after 6 days was no more than four times greater than that which inhibited growth for 24 hours. Viomycin sulfate thus appeared to maintain the major part of its antibacterial activity for at least 6 days in broth cultures at initial pH 8.5 as at pH 7.1.

EMERGENCE OF RESISTANT MYCOBACTERIA

It was of interest to compare the relative rates of emergence of resistant strains of *M. tuberculosis* H37Rv and *Mycobacterium tuberculosis* ATCC 607 *in vitro* to streptomycin sulfate and viomycin sulfate. In serial dilutions of each antibiotic (see table 3, methods 4, 4a), the minimum concentration totally inhibiting growth was observed. The contents of a tube showing significant growth of the organism (one or two tubes removed from that showing complete bacteriostasis) were ground with glass beads and another set of serial dilution tubes of the antibiotic was inoculated with 0.1 ml. of the ground suspension, followed by incubation for the stated period and repeated transfers to fresh serial dilution series in similar fashion.

In a parallel experiment with viomycin sulfate and streptomycin sulfate against H37Rv, the following bacteriostatic concentrations in γ per ml. were observed at the respective transfers: for viomycin sulfate: (*1*) 6.25, (*2*) 6.25, (*3*) 25, (*4*) 50, (*5*) >100; for streptomycin sulfate: (*1*) 0.39, (*2*) 0.78, (*3*) 3.12, (*4*) 3.12, (*5*) 6.25, (*6*) >100. In two parallel experiments with these antibiotics against

Mycobacterium tuberculosis ATCC 607, the following data were collected in the respective experiments: for viomycin sulfate, (*1*) 1.56, 1.56; (*2*) 1.56, 3.12; (*3*) 3.12, 3.12; (*4*) 6.25, 3.12; (*5*) 12.5, 3.12; (*6*) 12.5, 6.25; (*7*) 6.25, 25; (*8*) 12.5, 25; (*9*) 25, 50; (*10*) 50, —; (*11*) 100, —; (*12*) 100, —; for streptomycin sulfate, (*1*) 0.39, 0.39; (*2*) 0.78, 0.78; (*3*) 3.12, 25; (*4*) >100, >100.

These data indicate that the relative rates of emergence of resistance of H37Rv to viomycin and streptomycin are probably not significantly different. The rate of emergence of resistant strains of *Mycobacterium tuberculosis* ATCC 607 to viomycin, however, appears to be somewhat slower than to streptomycin. Resistance to streptomycin of both strains of *Mycobacteria* and resistance to viomycin of H37Rv appear to emerge suddenly. Emergence of resistance of *Mycobacterium tuberculosis* ATCC 607 to viomycin appears to be gradual. Resistance of both strains to both antibiotics appears to be maintained through many transfers in antibiotic-free medium.

A similar comparison of rates of emergence of resistance of the above obtained viomycin-resistant strain of *Mycobacterium tuberculosis* ATCC 607 to streptomycin and of the streptomycin-resistant strain of *Mycobacterium tuberculosis* ATCC 607 to viomycin was made. In both cases, the rate of emergence to the second antibiotic proceeded at the same rate as had been observed for the parent sensitive strain to the respective antibiotic.[3]

SUMMARY

Streptomyces floridae, the source of viomycin, was active on agar or in broth against various bacteria, notably the virulent human H37Rv strain of *Mycobacterium tuberculosis* var. *hominis*. Purified viomycin sulfate was bacteriostatic for this strain of tubercle bacilli at concentrations of 2.5 to 12.5 γ per ml. Other gram-positive and gram-negative bacteria and two actinomycetes were inhibited only by larger and variable amounts of the antibiotic. The fungi, protozoa, and viruses tested were not susceptible.

The antibacterial activity of viomycin sulfate tended to increase with increasing initial pH in the range between pH 7.1 and 8.5, and the major part of this activity was maintained in broth cultures for at least 6 days.

Streptomycin-resistant strains derived from H37Rv and *Mycobacterium tuberculosis* ATCC 607 were as sensitive to viomycin as were the respective parent strains. The rates of emergence of resistance of H37Rv to viomycin and streptomycin were probably not significantly different. The rate of emergence of resistant strains of *Mycobacterium tuberculosis* ATCC 607 to viomycin appeared to be somewhat slower than to streptomycin.

SUMARIO

Actividad Antimicrobiana del Streptomyces Floridae y de la Viomicina

El *Streptomyces floridae*, del cual procede la viomicina, se mostró activo en agar o en caldo contra varias bacterias, y notablemente contra la cepa humana

[3] Tested by A. C. Bratton, Jr., F. Montross, and Robert Rathbun of Parke, Davis & Company.

virulenta H37Rv del *Mycobacterium tuberculosis* var. *hominis*. El sulfato de viomicina purificado resultó bacteriostático para dicha cepa a concentraciones de 12.5 a 2.5 γ por ml. de caldo. Otras bacterias gram-positivas y gram-negativas y dos actinomicetos fueron inhibidos solamente por cantidades mayores y variables del antibiótico. Los hongos, protozoos y virus comprobados no resultaron susceptibles.

La actividad antibacteriana del sulfato de viomicina tendió a aumentar con un pH inicial de 7.1 a 8.5, manteniéndose la mayor parte de dicha actividad en cultivos en caldo por lo menos seis días.

Las cepas estreptomicinorresistentes derivadas del H37Rv y el *Mycobacterium* ATCC 607 fueron tan sensibles a la viomicina como las respectivas cepas matrices. Las proporciones de aparición de resistencia a la viomicina y la estreptomicina en el H37Rv no mostraron probablemente mayor diferencia. La velocidad de la aparición de cepas resistentes de ATCC 607 fué aparentemente algo menor con la viomicina que con la estreptomicina.

RESUME

Activité antimicrobienne de Streptomyces floridae et de la Viomycine

Streptomyces floridae, source de la viomycine, s'est montrée active sur agar ou dans le bouillon contre diverses bactéries, notamment sur la souche humaine virulente H37Rv de *Mycobacterium tuberculosis* var. *hominis*. Le sulfate de viomycine purifié s'est montré bactériostatique pour cette souche aux concentrations de 12,5 à 2,5 γ par ml. de bouillon. Des doses plus élevées et variables de l'antibiotique ont été nécessaires pour obtenir l'inhibition d'autres bactéries Gram-positives et Gram-négatives et de deux actinomycètes. Les champignons, protozoaires et virus ne se sont pas montrés sensibles à l'action du sulfate de viomycine.

Dans un milieu initial pH 7,1–8,5 l'activité antibactérienne du sulfate de viomycine tend à s'accroître; la majeure partie de cette activité s'est maintenue dans les cultures sur bouillon pendant au moins six jours.

Les souches streptomycino-résistantes dérivées de H37Rv et de *Mycobactérium* ATCC 607 se sont montrées aussi sensibles à l'action de la viomycine que leurs souches mères respectives. La vitesse d'apparition de la résistance de la souche H37Rv à la viomycine et à la streptomycine ne paraît pas avoir été sensiblement différente. La vitesse de l'apparition de souches résistantes ATCC 607 à la viomycine paraît avoir été un peu moins rapide qu'avec la streptomycine.

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THE ACTIVITY OF VIOMYCIN AGAINST MYCOBACTERIUM TUBERCULOSIS AND OTHER MICROORGANISMS IN VITRO AND IN VIVO[1]

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INTRODUCTION

In recent communications (1, 2), the isolation of viomycin, a new antimicrobial agent, has been reported. Viomycin is a tuberculostatic agent highly active against both streptomycin-sensitive and streptomycin-resistant strains of *M. tuberculosis* (3, 4). It is effective both *in vitro* and *in vivo*. The studies described herein were designed to evaluate more extensively the breadth of the antimicrobial activity of viomycin, its tuberculostatic activity *in vitro*, and its chemotherapeutic efficacy against experimental infections in animals.

Materials and Methods

Partially purified and crystalline preparations of viomycin sulfate[2] were used throughout the study of this new agent. The unit of activity of the preparations was defined as one microgram of the pure base, and the activities of the salts are stated throughout in terms of the equivalent weight of pure viomycin base.

Sensitivity to viomycin: The methods used to evaluate the effect of viomycin against the nonacid-fast microorganisms have been described in detail elsewhere (5).

In vitro studies on the tuberculostatic action of viomycin have been performed with a graded serial dilution technique, using the Dubos-Davis liquid medium containing 0.02 per cent Tween 80 and 0.5 per cent albumin. Unless otherwise specified, the sensitivity of an organism was accepted as the lowest concentration of antibiotic necessary to inhibit growth completely for a period of 12 days at 37°C.

Resistance to viomycin: The rate at which strains resistant to viomycin emerge from predominantly sensitive strains was determined by cultivation of the organisms in various concentrations of viomycin in Tween-albumin medium. In the initial transfer, the concentrations of viomycin ranged from slightly more to slightly less than the previously determined minimal inhibiting concentration. The inoculum consisted of 0.1 ml. of a 4- to 6-day culture, diluted to the equivalent of 92 to 94 per cent transmission (Photovolt Lumetrom No. 400) per 2.5 ml. of medium. Thereafter, 0.1 ml. from the highest concentration of antibiotic showing light (1+) growth was seeded into broth containing increasing amounts of antibiotic, the process being repeated until the strain was resistant to 1,000 γ per ml. A 14- to 18-day incubation period was used throughout to allow time for the more slowly growing, more resistant cells to multiply.

Chemotherapeutic action of viomycin: All animal experiments utilized either the C57 strain or a dba substrain of pigmented mice. Animals were infected intracerebrally with

[1] From the Research Laboratories, Chas. Pfizer & Co., Inc., Brooklyn, New York.

[2] All of the viomycin used throughout this study was prepared by the Departments of Biochemical and Chemical Research and Development, Chas. Pfizer & Co., Inc. We are indebted especially to Dr. B. A. Sobin and Dr. P. P. Regna for supplies during the early part of the study.

a 10-day culture of the H37Rv strain of *M. tuberculosis* grown in the Dubos liquid medium (6), and were treated daily by the subcutaneous or oral route, beginning usually on the day of infection and continuing for one month. The ability of viomycin to suppress infection was measured in all instances in terms of the average survival time and the percentage survival of treated animals, in contrast to untreated control animals infected and observed simultaneously. The presence of tuberculosis at the time of death or of sacrifice was determined in all instances by gross examination of the lesions and by culture, and in some instances by microscopic examination of histopathologic sections.

Pharmacology of viomycin: Observations were made on the toxicity of viomycin in mice and rats and on its rate of absorption and excretion in dogs, using the methods described previously for terramycin (5, 7). In the absorption and excretion studies, measurement of the antibiotic in serum and urine was made by bioassay, with *K. pneumoniae* as the test organism and crystalline viomycin sulfate as standard.

TABLE 1

The Tuberculostatic Activity of Viomycin

STRAIN OF M. TUBERCULOSIS	MINIMAL INHIBITORY CONCENTRATION	
	Viomycin	Streptomycin
	γ *per ml*	γ *per ml.*
H37Rv		
Isolate #1075	2.8	>2,500
#1337	1.0	2,500
#1855	5.2	<0.25
#2290	2.8	<0.25
Freshly Isolated		
Mund	1.9	>1,000
Nit	1.0	6.3
Bel	2.8	25
Mun	2.8	<0.25
Sta	5.6	>5,000
Mar	2.8	1.25
Mos	2.8	0.25

Tween-albumin medium containing 0.02 per cent Tween 80 and 0.5 per cent albumin was used throughout.

IN VITRO ACTIVITY

Sensitivity to viomycin: The sensitivity to viomycin of a variety of microorganisms was tested by the methods described. Test strains of *M. tuberculosis* included both streptomycin-sensitive and streptomycin-resistant strains. One to 5 γ of viomycin sulfate per ml. of medium were sufficient to inhibit growth of all strains of tubercle bacilli tested, whereas much higher concentrations were necessary for inhibition of the nonacid-fast microorganisms (tables 1 and 2). No activity was observed against 22 strains of plant and human pathogenic fungi.

The action of viomycin on streptomycin-resistant as well as streptomycin-sensitive strains of *M. tuberculosis* suggested its potential usefulness in combina-

tion with streptomycin and other tuberculostatic agents. Preliminary *in vitro* studies were carried out to evaluate this potentiality. Subinhibitory concentrations of viomycin, equivalent to one-half to one-tenth of the amount required for inhibition of growth, frequently, although not invariably, reduced significantly the concentration of streptomycin required to inhibit growth (table 3).

TABLE 2

Antibacterial Action of Viomycin in Vitro

MICROORGANISMS	MINIMAL INHIBITORY CONCENTRATION OF VIOMYCIN
	γ/ml.
Str. hemolyticus (C203Mv)	80
D. pneumoniae (I/230)	40
E. coli	40
S. typhosa	159
S. typhosa (streptomycin-resistant strains)	318 and >636
Salmonella sp.	159
Staph. aureus (H)	40
A. aerogenes	40
S. paratyphosa	80
Proteus vulgaris	>636
Klebsiella pneumoniae	20
Brucella bronchiseptica	80
Pseudomonas pyocyanea	>636
B. subtilis	20
H. influenzae	12

TABLE 3

The Tuberculostatic Activity of Viomycin Alone and in Combination with Other Antimicrobial Agents

STRAIN	CONCENTRATION OF VIOMYCIN	SENSITIVITY TO:						
		VM*	SM	TM	NM	AM	CM	PN
	(γ/ml.)	(γ/ml.)						
H37Rv #2678	0	3.1	0.6	10	1.6	20	15	320
	0.3		0.2	3.1	0.8	10	10	320
	0.6				0.6	2.5	10	320
	1.3		0.02	0.63	0.08	0.6	0.6	2.5

* VM indicates viomycin; SM, streptomycin; TM, terramycin; NM, neomycin; AM, aureomycin; CM, chloramphenicol; PN, penicillin.

Subinhibitory concentrations of terramycin, the tuberculostatic activity of which is discussed in detail elsewhere (3, 4, 8, 9), likewise decreased by at least one-half the amount of viomycin necessary for inhibition of growth (table 4).

Resistance to viomycin: Viomycin is bacteriostatic in nature and, like streptomycin, rarely exerts a bactericidal effect. It further resembles streptomycin in the pattern in which resistance becomes manifest. Under the experimental

conditions used, the number of transfers in subinhibitory concentrations of viomycin required to increase the resistance of the strains of tubercle bacilli tested was invariably more than with streptomycin (table 5).

CHEMOTHERAPEUTIC ACTION

The ability of viomycin to suppress experimental tuberculous infections in mice was determined as described above. Doses of 0.5 mg. or more of viomycin

TABLE 4

The Tuberculostatic Activity of Viomycin in Combination with Terramycin

CONCENTRATION OF TERRAMYCIN (γ/ml.)	SENSITIVITY TO VIOMYCIN (γ/ml.)			
	Strain H37Rv # 2678	Strain Mun.	Strain Mund.	Strain Sta.*
0	3.1	5.0	5.0	3.1
0.12	3.1	3.1		
0.25	2.5	3.1	5.0	2.5
0.63	2.5		5.0	3.1
1.25	1.6	3.1	1.6	
2.50	1.6	1.6	1.2	1.2

* Streptomycin-resistant strain.

TABLE 5

A Comparison of the Rate of Emergence of Resistance to Viomycin and Streptomycin of Five Strains of Tubercle Bacilli

STRAIN	ANTIBIOTIC	INCREASE IN RESISTANCE: NUMBER OF TRANSFERS REQUIRED				
		2X	5X	10X	100X	1,000X
$H_{37}Rv$ (2290)	Viomycin	3	4		5	
	Streptomycin		2	3	4	5
McV	Viomycin	9		11	12	
	Streptomycin	2	3	7	8	9–10
Mos.	Viomycin	14				
	Streptomycin	2		3	6	7
Mund.	Viomycin	7		8	11	
	Streptomycin					
Mun.	Viomycin	5	12		13	
	Streptomycin	2	3	4	5–6	6–7

daily were sufficient to increase significantly the average survival time and the percentage survival of infected animals, as calculated at 90 to 180 days following infection, above that of untreated controls. Satisfactory chemotherapeutic results likewise were obtained when treatment was started 8 days following, rather rather than on, the day of infection (table 6).

Viomycin, even in high dosages, failed to protect mice against experimental infections due to *Streptococcus hemolyticus*, *D. pneumoniae*, or *Pseudomonas pyocyanea*, but showed definite chemotherapeutic activity against *K. pneumoniae*, *E. typhosa*, and *Proteus vulgaris* under comparable conditions. Viomycin was effective against these organisms when administered orally as well as subcutaneously, provided a sufficiently large dosage was used (table 7).

PHARMACOLOGY

Preliminary observations on impure preparations of viomycin indicated that it is readily absorbed following parenteral and oral administration (10). The acute intravenous LD_0 was found to be equivalent to 2.4 mg. of pure viomycin per 20-gram mouse, while its acute intravenous LD_{50} was equivalent to about

TABLE 6

The Chemotherapeutic Action of Viomycin in Experimental Tuberculous Infections in Mice (Strain H37Rv)

	DAILY DOSAGE (MG. PER MOUSE)	NUMBER OF MICE	ROUTE OF ADMINISTRATION	AVERAGE SURVIVAL TIME (DAYS)				PERCENTAGE SURVIVAL			
				Days of Observation				Days of Observation			
				30	60	90	180	30	60	90	180
Treatment started on day of infection	4.18	30	Subcutaneous	30	58	83	145	97	83	80	63
	3.52	20	Subcutaneous	30	57	84	165	90	90	90	90
	2.09	20	Subcutaneous	30	58	82	124	100	85	60	40
	1.76	20	Subcutaneous	28	49	64	77	80	50	40	10
	1.00	38	Subcutaneous	27	53	70	105	87	77	52	34
	0.50	20	Subcutaneous	29	57	84	146	95	90	70	60
Treatment started 8 days following infection	3.52	10	Subcutaneous	28	56	82	105	90	90	90	20
	1.76	10	Subcutaneous	27	49	70	125	80	70	70	60
	0.00	50	Subcutaneous	26	44	60	79	76	50	32	10

3.3 mg. of pure viomycin per 20-gram mouse. When dosages equivalent to 2.4 to 4.2 mg. of pure viomycin were administered subcutaneously to 20-gram mice once or twice daily for periods of 5 days, no toxicity was observed. In addition, chronic toxicity studies in rats revealed no renal damage following administration of 12 to 36 mg. of viomycin per 100-gram rat daily for 3 to 5 days.

More extensive studies on the toxicity of viomycin in animals have been carried out by P'an and his associates (11) who have observed no toxicity in cats, dogs, or other species of animal with doses equivalent to 50 mg. or less of pure viomycin per kg. of body weight, administered daily for prolonged periods of time. Studies by Muschenheim and his associates, to be reported in detail at a later date, indicate that in this dosage (50 mg. per kg. of body weight per day) viomycin can be administered to humans for as long as 3 to 6 months without undue risk of toxic reaction (12).

TABLE 7

The Chemotherapeutic Action of Viomycin on Experimental Infections of Mice Other than Tuberculosis

STRAIN	DOSAGE REGIMEN*	ROUTE OF ADMINISTRATION	CHEMOTHERAPEUTIC RESPONSE CD_{50} (19/20 CONFIDENCE LIMITS)	
			Observation Period	
			48 hours	7 days
			mg. per single dose per 20-gram mouse	
K. pneumoniae (No. 1)	Twice daily for 2½ days	Subcutaneous	0.26 (0.03–0.47)	0.26 (0.03–0.47)
	Single dose	Subcutaneous	0.33 (approx.)	0.6 (0.17–2.1)
	Twice daily for 2½ days	Oral	0.25 (approx.)	0.25 (approx.)
	Single dose	Oral	0.44 (approx.)	0.44 (approx.)
E. typhosa (St.)	Twice daily for 2½ days	Subcutaneous	0.26 (approx.)	0.4 (0.2–0.8)
	Single dose	Subcutaneous	0.62 (0.56–0.68)	0.62 (0.56–0.68)
	Twice daily for 2½ days	Oral	1.4 (0.28–7.0)	2.3 (0.82–6.44)
	Single dose	Oral	0.88 (approx.)	0.88 (approx.)
Proteus vulgaris	Twice daily for 2½ days	Subcutaneous	0.22 (0.17–0.29)	0.24 (0.19–0.3)
	Single dose	Subcutaneous	5.8 (3.9–8.7)	5.8 (3.2–10.44)
Pseudomonas pyocanea	Twice daily for 2½ days	Subcutaneous	100 (approx.)	100 (approx.)
D. pneumoniae (I/230)	Twice daily for 2½ days	Subcutaneous	2.6 (approx.)	>3.5†
Str. hemolyticus (C_{203}Mv)	Twice daily for 2½ days	Subcutaneous	2.8 (1.9–4.2)	4.2 (approx.)
	Single dose	Subcutaneous	1.6 (0.94–2.72)	2.1 (approx.)

* A minimum of 10 mice for each dose tested and 3 to 6 test doses per dosage regimen were used for the evaluation of viomycin against each organism. Animals receiving viomycin twice daily were treated for 2½ days (total 5 doses) beginning one-half hour after infection. Other animals received a single dose one half-hour after infection. The CD_{50} (19/20 Confidence Limits) values were calculated according to the method of Litchfield and Wilcoxon (13).

† No protection with dosages of 3.5 mg. per 20-gram mouse.

DISCUSSION

It was recognized early in the evaluation of viomycin that this antibiotic resembles streptomycin in many of its biologic properties. It differs from streptomycin in that it is active primarily against acid-fast bacilli, with relatively little activity *in vitro* against gram-negative microorganisms. Moreover, vio-

mycin is also active against streptomycin-resistant strains of tubercle bacilli. Its ability to control experimental infections in mice due to *E. typhosa*, *Proteus vulgaris*, and *K. pneumoniae* illustrates the discrepancies which may exist between *in vitro* and *in vivo* testing. Its activity against *M. tuberculosis*, however, is confirmed by its striking ability to suppress the course of experimental tuberculous infections in mice and in guinea pigs, as illustrated respectively by the present report and the report of Steenken and Wolinsky (4, 9).

Viomycin is readily absorbed following parenteral or oral administration and is an antibiotic of relatively low toxicity. Extensive clinical studies will be required to evaluate the toxicity of viomycin in man or its therapeutic efficacy in tuberculosis. Conceivably, its main value may lie in its use in combination with other antimicrobial agents such as streptomycin. The discovery of another agent active on streptomycin-resistant strains of tubercle bacilli offers another potential tool for use in combined therapy.

SUMMARY

Viomycin is a new antibiotic active *in vitro* against streptomycin-sensitive and streptomycin-resistant strains of tubercle bacilli. It is capable of exerting a marked suppressive effect on tuberculous infections in experimentally inoculated mice. It is a substance of moderately low toxicity and offers promise as a potential chemotherapeutic agent in man.

SUMARIO

La Actividad de la Viomicina contra el Mycobacterium Tuberculosis y Otros Microorganismos, in Vitro e in Vivo

La viomicina es un nuevo antibiótico que se ha mostrado activo *in vitro* contra las cepas estreptomicinorresistentes y estreptomicinosensibles de los bacilos tuberculosos. También es capaz de suprimir en forma notable las infecciones tuberculosas en los ratones infectados experimentalmente. La sustancia posee una toxicidad moderadamente baja y da esperanzas como potencial agente quimioterapéutico en el hombre.

RESUME

Activité de la Viomycine in vitro et in vivo contre Mycobacterium Tuberculosis et d'autres bactéries

La viomycine est un nouvel antibiotique actif *in vitro* contre les souches streptomycino-résistantes et streptomycino-sensibles du bacille tuberculeux. Elle est capable de combattre dans une large mesure l'infection tuberculeuse chez la souris inoculée. C'est une substance de toxicité relativement faible et qui offre des promesses en tant qu'agent chimiothérapique futur pour l'homme.

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THE EFFECT OF VIOMYCIN IN VITRO AND IN VIVO ON MYCOBACTERIUM TUBERCULOSIS[1,2]

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INTRODUCTION

In the course of a cooperative program designed to test the antibiotic producing potentialities for *Mycobacterium tuberculosis* var. *hominis* of a large number of actinomycetes[3] using a streak-plate test method (1), a culture (designated A-5014) was encountered which produced zones of inhibition of the growth of both streptomycin-sensitive and -resistant strains of the virulent human-type tubercle bacillus H37Rv. Subsequent collaborative investigation revealed that the active principle was present in shake culture filtrates and apparently differed from any antibiotic previously reported (2, 3).

Since that time the bacteriostatic activity of several samples[3] of the crystalline antibiotic from active shake culture filtrates of this actinomycete culture have been tested both *in vitro* and *in vivo* on *Mycobacterium tuberculosis* var. *hominis*. Recently Hobby (4), using mice, and Steenken (5), using guinea pigs, reported on the *in vivo* tuberculostatic activity of viomycin. Subsequent investigation has shown that the above antibiotic and viomycin are identical, and the name, viomycin, is accordingly employed in this report, which summarizes a study of the effect of viomycin *in vitro* and *in vivo* on *M. tuberculosis*.

Materials and Methods

The *in vitro* tuberculostatic tests were performed, using the liquid modified Proskauer and Beck synthetic medium both with and without 10.0 per cent beef serum, as described previously for the testing of tuberculostatic activity of streptomycin and other substances (6, 7, 8). The antibiotic was sterilized by filtration through sintered glass filters after it had been dissolved in the synthetic medium. Appropriate dilutions in the liquid media then were prepared aseptically.

RESULTS

In table 1 is shown the sensitivity to viomycin sulfate and to streptomycin sulfate of 18 strains of virulent tubercle bacilli. All of these except H37Rv and 97R had been isolated recently from patients suffering from active pulmonary tuberculosis and were tested following two subcultures in Tween-albumin medium. The values for the sensitivity to streptomycin given in table 1 were obtained from the results of our routine streptomycin sensitivity test as performed on egg-yolk nutrient agar medium (9). Previous studies have shown that in the

[1] From the Department of Bacteriology, Northwestern University Medical School, Chicago 11, Illinois.

[2] Aided by a research grant from Parke, Davis & Company, Detroit, Michigan.

[3] Submitted by Research Laboratories, Parke, Davis & Company, Detroit, Michigan.

majority of cases the sensitivity of tubercle bacilli to streptomycin is the same when determined in serum synthetic medium and in the egg-yolk agar medium (9). The data indicate that streptomycin-sensitive tubercle bacilli are approximately from one-half to one-fourth as sensitive to viomycin *in vitro* as they are to streptomycin. Streptomycin-resistant tubercle bacilli, however, are just as sensitive to viomycin as the streptomycin-sensitive strains.

The results of preliminary studies also indicate that virulent human-type tubercle bacilli will become resistant to viomycin fairly rapidly *in vitro*. To date, 2 strains have become resistant to 250.0 γ of viomycin per ml. following 5 and

TABLE 1

In Vitro Bacteriostatic Activity of Viomycin Sulfate for Virulent Tubercle Bacilli

STRAIN	SENSITIVITY TO STREPTOMYCIN SULFATE IN γ PER ML.*		SENSITIVITY TO VIOMYCIN SULFATE IN γ PER ML.*	
			Proskauer and Beck synthetic medium	Proskauer and Beck synthetic medium plus 10.0 per cent beef serum
1448	>3.0	<10.0	3.12	6.25
2016	>10.0	<100.0	6.25	12.5
1438		<3.0	6.25	12.5
1427	>3.0	<10.0	3.12	6.25
1451		3.12	3.12	6.25
1452		3.12	3.12	12.5
1461		<3.0	3.12	6.25
1462		<3.0	3.12	6.25
1464		<3.0	3.12	6.25
1458		25.0	3.12	6.25
1423		>1,000.0	3.12	6.25
1424		>1,000.0	3.12	12.5
1439		>1,000.0	3.12	12.5
1450	>25.0	<50.0	3.12	12.5
1459		>100.0	3.12	6.25
1460		100.0	3.12	12.5
97R		>1,000.0	3.12	6.25
H37Rv		1.56	3.12	6.25

* Least amount of streptomycin sulfate and viomycin sulfate which completely inhibited growth.

6 transfers, respectively, in Tween-albumin medium containing low concentrations of viomycin.

The determination *in vivo* of the effectiveness of viomycin for the suppression of experimental tuberculous infection of mice was performed as described in previous publications from this laboratory (10, 11). The viomycin sulfate was administered subcutaneously either once or twice daily to mice infected intravenously with virulent tubercle bacilli. The virulent H37Rv strain was employed in all experiments except one in which the streptomycin-resistant strain 97R was used.

In a preliminary experiment, 5 mice were treated for a period of twenty-one

days with 8.0 mg. per day given in 2 doses of 4.0 mg. approximately eight hours apart. The mice were infected intravenously with 0.2 mg. wet weight of the H37Rv strain. In table 2 the results of this experiment are presented. For comparative purposes, 2 groups of mice treated with streptomycin were also included. The number of animals employed was too small to permit any real evaluation of the effectiveness of viomycin for the suppression of experimental tuberculosis of mice. The results suggested, however, that viomycin, in the dosage employed, was as effective as the amounts of streptomycin administered, and further larger scale experiments seemed warranted.

Results of a larger experiment are given in table 3. Due to an appreciable decrease in virulence of our H37Rv strain, in this experiment the mice were infected intravenously with 1.0 mg. of the H37Rv strain in order to produce a mortality rate in the controls comparable to that previously obtained with the 0.2 mg. infecting doses. A 1.0 mg. inoculum of the 97R strain was also employed.

TABLE 2

The Effect of Viomycin Sulfate on Experimental Tuberculosis in the Mouse

ANTIBIOTIC	DOSAGE OF DRUG PER MOUSE	NUMBER OF MICE	MORTALITY	AVERAGE WEIGHT LOSS OR GAIN	AVERAGE SURVIVAL TIME	AVERAGE AMOUNT OF GROSS PULMONARY TUBERCULOSIS
	mg.		*per cent*	*per cent*	*days*	
Viomycin sulfate	8.0	5	0.0	+2.8	21.0	1.0+
Streptomycin sulfate	3.0	5	0.0	+2.8	21.0	1.0+
Streptomycin sulfate	1.5	5	0.0	+15.0	21.0	1.8+
Controls (untreated)	0.0	5	80.0	−35.6	19.8	4.0+

1+ = 0- 10% of lung involved with tuberculosis.
2+ = 10- 25% of lung involved with tuberculosis.
3+ = 25- 50% of lung involved with tuberculosis.
4+ = 50-100% of lung involved with tuberculosis.

The viomycin sulfate and the streptomycin sulfate were administered subcutaneously once daily. The experiment was terminated at twenty-three days by sacrificing the surviving animals.

An appreciable amount of toxicity was encountered with the 6, 10, and 12 mg. doses of viomycin. Those mice which died early, due to toxicity and without significant amounts of grossly visible pulmonary tuberculosis, have been excluded from the table, thus giving us a truer picture of the effect of these concentrations of viomycin on the suppression of the disease. While the data in table 3 indicate that comparable doses of viomycin and streptomycin have an equal suppressive effect on the tuberculous process, we must keep in mind that the method employed is far from quantitative and appreciable differences in therapeutic effectiveness might not be noted.

A superficial examination of the data on the amount of gross pulmonary tuberculosis in the mice infected with strain 97R (streptomycin-resistant) might suggest that viomycin was no more effective than streptomycin. These figures,

however, are misleading because those animals treated with streptomycin had an average survival time less than that of the controls. Although it cannot be assumed from these data that streptomycin exerted a deleterious effect on the infection produced by this resistant organism, apparently the infection was so overwhelming that there was insufficient time for development of the large

TABLE 3

The Effect of Viomycin Sulfate and Streptomycin Sulfate on Experimental Tuberculosis in the Mouse

ANTIBIOTIC	DOSAGE OF DRUG PER MOUSE	NUMBER OF MICE	MORTALITY	AVERAGE WEIGHT LOSS OR GAIN	AVERAGE SURVIVAL TIME	AVERAGE AMOUNT OF GROSS PULMONARY TUBERCULOSIS
Strain H37Rv						
	mg.		*per cent*	*per cent*	*days*	
Viomycin sulfate	12.0	8*	0.0	−4.6	23.0	1.9+
	6.0	13†	0.0	−1.7	23.0	2.2+
	3.0	19	0.0	+7.1	23.0	3.05+
	1.5	20	0.0	+10.6	23.0	3.6+
Streptomycin sulfate	3.0	20	0.0	+11.8	23.0	3.6+
	1.5	20	0.0	+13.0	23.0	3.7+
	0.75	20	0.0	+16.8	23.0	3.4+
	0.375	18	5.5	0.0	22.8	4.0+
Controls		20	80.0	−34.6	19.1	4.0+
Strain 97R (Streptomycin-resistant)						
Viomycin sulfate	10.0 9 days 6.0 14 days	12‡	0.0	−10.7	23.0	2.9+
Streptomycin sulfate	3.0	19	89.4	−23.2	16.4	2.6+
Controls		20	70.0	−24.1	19.2	3.7+

1+ = 0- 10% of lung involved with tuberculosis.
2+ = 10- 25% of lung involved with tuberculosis.
3+ = 25- 50% of lung involved with tuberculosis.
4+ = 50-100% of lung involved with tuberculosis.
* 12 mice which died early not included.
† 7 mice which died early not included.
‡ 8 mice which died early not included.

amount of grossly visible pathology usually seen. The mortality data bear this out; none of the viomycin-treated animals died of tuberculosis, whereas 89.4 per cent of the streptomycin-treated animals succumbed.

It would appear to be safe to conclude that viomycin had a pronounced suppressive effect on experimental murine tuberculosis, whether produced with streptomycin-sensitive or streptomycin-resistant human-type tubercle bacilli.

Since the completion of the above experiments, preparations of viomycin have been obtained which are relatively nontoxic, inasmuch as 12.0 mg. can be administered to mice subcutaneously daily for a period of 28 days without apparent ill effect. The therapeutic effectiveness in mice of these preparations is now being investigated by a more quantitative method based on that recommended by Baker, Schlosser, and White (12). While the details of these tests will be reported subsequently, it can be stated that these less toxic preparations also show a pronounced suppressive effect on the tuberculous process.

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VIOMYCIN IN EXPERIMENTAL TUBERCULOSIS[1,2]

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INTRODUCTION

The antibiotic, viomycin, was introduced at the Ninth Streptomycin Conference of the Veterans Administration in April 1950 (1, 2, 3, 4). It is the purpose of this report to present observations on the *in vitro* activity of this antibiotic against virulent tubercle bacilli and on its effect on early progressive tuberculosis in guinea pigs.

IN VITRO ACTIVITY OF VIOMYCIN

The ability of viomycin[3] to inhibit the growth of tubercle bacilli was tested in three different types of liquid media: the Tween-albumin medium of Dubos; Proskauer and Beck's synthetic medium; and Proskauer and Beck's medium with 10 per cent beef serum. The test strains employed were the virulent human strain, H37Rv, and a streptomycin-resistant strain derived from it. The inoculum into each tube was 0.1 cc. of a 10-day culture of the microorganisms in Tween-albumin medium.

The results are presented in table 1.

It may be noted from table 1 that after 14 days of incubation viomycin in concentration of 5.0 γ per cc. completely inhibited growth of both the streptomycin-sensitive and the streptomycin-resistant H37Rv microorganisms. The bacteriostatic effect of viomycin was not influenced by the presence of either Tween 80 or serum. An additional 7 days of incubation produced little change in the minimal inhibitory concentration except in the medium containing serum, where growth eventually appeared in the tube containing 5 γ per cc.

DRUG RESISTANCE

A viomycin-resistant strain of tubercle bacilli was obtained by serial transfers of strain H37Rv in Tween-albumin liquid medium containing gradually increased concentrations of viomycin. In this manner, a strain resistant to the action of 1,000 γ of viomycin per cc. was produced after 8 transfers. There was very little increase noted in the first 4 transfers, but thereafter each succeeding passage produced a rapid rise in resistance. Previous experience in this laboratory (5) has shown that H37Rv tubercle bacilli, when handled in this manner, become highly resistant to streptomycin after 6 transfers, and to neomycin after 7 transfers.

[1] From the Trudeau Laboratory of the Trudeau Foundation for the Clinical and Experimental Study of Pulmonary Disease, Trudeau, New York.

[2] This study was supported (in part) by a research grant from the National Institutes of Health, Public Health Service.

[3] Viomycin was kindly supplied by Chas. Pfizer & Co., Inc., Brooklyn, New York.

TUBERCULOSTATIC EFFECT OF VIOMYCIN IN VIVO

Twenty-nine tuberculin-negative guinea pigs, weighing between 500 and 800 gm., were infected subcutaneously with 0.13 mg. (dry weight) of H37Rv streptomycin-sensitive organisms. An additional 24 guinea pigs were infected in the same manner with the streptomycin-resistant H37Rv culture. Treatment was begun 26 days after infection, at which time all the animals demonstrated a positive skin reaction to 5 per cent Old Tuberculin.

The animals infected with the streptomycin-sensitive culture were treated as follows: 5 animals received streptomycin, 10 mg. once daily by intramuscular injection; 7 received viomycin, 20 mg. once a day; and 7 received 40 mg. once a day by intramuscular injection; 10 animals remained untreated. The animals infected with the streptomycin-resistant culture were treated as follows: 7 received viomycin, 20 mg. once daily; and 7, 40 mg. once daily by intramuscular injection; 10 animals remained untreated. All survivors in the group infected

TABLE 1

In Vitro Inhibiting Action of Viomycin on Tubercle Bacilli*

TEST STRAIN	MEDIUM	MINIMAL INHIBITORY CONCENTRATION (γ PER CC.)		
		10 days	14 days	21 days
H37Rv, sensitive to streptomycin	Tween-albumin	0.1	5.0	5.0
	Proskauer & Beck	5.0	5.0	5.0
	Proskauer & Beck with serum	5.0	5.0	10.0
H37Rv, resistant to streptomycin	Tween-albumin	0.1	5.0	5.0

* Viomycin sulfate—Lot 430-144A, 760 γ per mg.

with the streptomycin-resistant culture were sacrificed 74 days after infection, or after 48 days of treatment. The results are shown in table 2.

From the results recorded in table 2, it may be observed that the administration of viomycin markedly retarded the progression of the tuberculous infection. The animals in the untreated control group revealed far advanced, progressive tuberculous lesions in the lungs, liver, spleen, and lymph nodes. The animals treated with the higher dosage of viomycin showed very little disease in the lungs, liver, or spleen. Gross lesions in this group were confined mainly to the lymph nodes contiguous to the site of inoculation. The animals treated with the lower dosage of viomycin definitely exhibited more gross disease, which was still only approximately one-half the amount of tuberculosis seen in the control group.

The guinea pigs infected with the streptomycin-sensitive H37Rv culture were observed for an additional 38 days. The dosage for all viomycin-treated animals was increased to 80 mg. once daily, while the dosage for animals treated with streptomycin remained at 10 mg. once daily. All survivors were sacrificed after

TABLE 2

Effect of 48 Days of Viomycin Therapy on Experimental Tuberculosis of Guinea Pigs Infected with the Streptomycin-resistant H37Rv Strain

TREATMENT	DAYS OF TREATMENT	NUMBER SURVIVING 74 DAYS/TOTAL NUMBER IN GROUP	AVERAGE INDEX OF TUBERCULOSIS*				
			Lungs	Liver	Spleen	Lymph Nodes	Total
None (Controls)	0	7/9†	2.4	2.7	2.9	3.4	11.4
Viomycin‡ intramuscularly, 20 mg. once daily	48	7/7	1.0	1.4	1.1	2.2	5.7
Viomycin intramuscularly, 40 mg. once daily	48	7/7	0.8	0.65	0.6	1.4	3.5

* Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of 0 to 4 according to the amount of gross tuberculous disease. Maximum total for each animal was, therefore, 16. Index of tuberculosis is the average total for each group.

† One animal died prematurely of intercurrent infection, and is not included in the table.

‡ Lots of viomycin sulfate used were: 430-144A, 760 γ/mg.; 636-267A, 880 γ/mg.; and N 499003.

TABLE 3

Effect of 86 Days of Viomycin Therapy on Experimental Tuberculosis of Guinea Pigs Infected with the Streptomycin-sensitive H37Rv Strain

TREATMENT	DAYS OF TREATMENT	NUMBER SURVIVING 112 DAYS/TOTAL NUMBER IN GROUP	AVERAGE INDEX OF TUBERCULOSIS*				
			Lungs	Liver	Spleen	Lymph Nodes	Total
None (Controls)	0	1/7†	3.2	3.1	3.4	3.5	13.2
Viomycin intramuscularly, 20 mg. once daily for 48 days; 80 mg. once daily for 38 days	86	5/5‡	0.7	0.5	0	1.8	3.0
Viomycin intramuscularly, 40 mg. once daily for 48 days; 80 mg. once daily for 38 days	86	7/7	0	0	0.1	1.8	1.9
Streptomycin intramuscularly, 10 mg. once daily	86	5/5	0	0	0.2	1.8	2.0

* Each organ (lungs, liver, spleen, lymph nodes) was assigned a value of 0 to 4 according to the amount of gross tuberculous disease. Maximum total for each animal was, therefore 16. Index of tuberculosis is the average total for each group.

† Three animals died prematurely of intercurrent infection, and are not included in the table.

‡ Two animals died prematurely of intercurrent infection and are not included in the table.

112 days of infection and a total of 86 days of treatment. The average amounts of tuberculosis seen at this time are shown in table 3.

The results as shown in table 3 indicate that viomycin, in the high dosages employed, produced a tuberculostatic effect in the guinea pigs entirely com-

parable to streptomycin, 10 mg. a day. Aside from the lesions in the lymph nodes contiguous to the site of inoculation, the only evidences of gross tuberculosis in the animals treated with viomycin were tiny, gray, pin-point areas in some of the lungs and livers.

Microscopic sections were prepared from the organs of 6 guinea pigs treated for 86 days with viomycin, chosen at random from among the 12 surviving

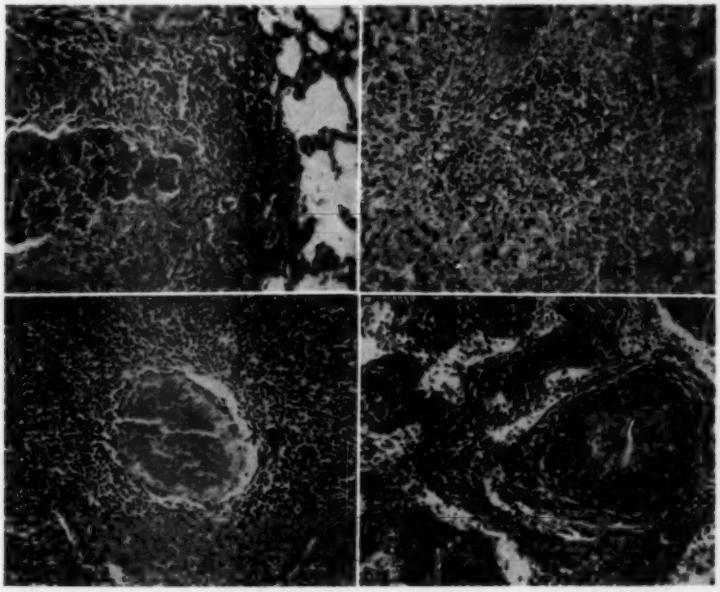

FIG. 1. Microscopic sections from organs of guinea pigs treated with viomycin for 86 days: a. (Upper left) Lungs—A tuberculous focus showing necrotic central portion surrounded by epithelioid cells, giant cells, and lymphocytes. There is some peripheral fibrosis. (×200); b. (Upper right) Liver—Small confluent epithelioid tubercles adjacent to a central vein. The predominant cell type is the epithelioid cell. The lesions are surrounded by a few lymphocytes. (×200); c. (Lower left) Spleen—A necrotic tuberculous lesion in a germinal center. There is no recognizable inflammatory cellular reaction about this focus and only slight evidence of scar formation. (×200); d. (Lower right) Hepatic lymph node—Note the small epithelioid cell tubercle with a central multinucleated giant cell. (×200).

animals. The findings on microscopic examination of these sections were as follows:

Lungs: The lungs of several of the animals displayed scattered areas of lymphoid tissue. These lymphoid aggregates have been observed in normal guinea pigs. In one animal an epithelioid cell tubercle with central caseation was noted (figure 1a). This was the only such lesion found in the lungs of any of the guinea pigs studied by microscopic examination.

Livers: No lesions were seen in 3 animals. Slight fatty infiltration was noted

in one. In another, there was a small lesion in a portal space, made up chiefly of epithelioid cells and lymphocytes, with a small amount of fibrous tissue. One guinea pig showed two epithelioid foci (figure 1b).

Spleens: In 4 of the animals the spleen showed hyperplasia and a few clusters of epithelioid cells. Two guinea pigs had from one to three small necrotic lesions without inflammatory reaction (figure 1c).

Hepatic lymph nodes: No microscopic lesions were seen in 2 of the animals. One showed a fibrocellular tubercle without necrosis; one had two small, irregular epithelioid tubercles; another demonstrated advanced caseous disease; and a single epithelioid tubercle (figure 1d) was seen in another.

DISCUSSION

The administration of viomycin in doses as high as 150 mg. per kg. per day produced no visible toxic effects in guinea pigs. The animals did not lose weight, and did not appear ill. There was no local tissue damage at the sites of drug injections.

The guinea pigs treated with 70 to 140 mg. per kg. of viomycin per day revealed the presence of small, tuberculous abscesses at the original site of inoculation in the inguinal region. This same phenomenon has been consistently observed in this laboratory in subcutaneously infected guinea pigs treated with streptomycin. It would appear, therefore, that neither of these drugs is able to eradicate a walled-off tuberculous abscess.

In these experiments it was demonstrated that in experimental guinea pig tuberculosis viomycin produced beneficial results entirely comparable to those obtained with streptomycin. To produce this effect, however, it was necessary to administer approximately four times the amount of viomycin as streptomycin.

SUMMARY

Viomycin inhibited the growth of virulent tubercle bacilli (strain H37Rv) in a concentration of 5 γ per cc., and was as active against streptomycin-resistant as against streptomycin-sensitive microorganisms. Its inhibitory effect was not antagonized by the presence of serum.

A strain of tubercle bacilli highly resistant to the action of viomycin *in vitro* was produced after 8 transfers in liquid medium containing increased concentrations of the drug.

Viomycin was highly active in retarding the progress of experimental tuberculosis in guinea pigs. It was just as effective in disease produced by streptomycin-resistant as by streptomycin-sensitive tubercle bacilli.

The intramuscular administration of 40 mg. of viomycin once daily produced a beneficial effect in guinea pigs approximately equal to that obtained with intramuscular administration of 10 mg. of streptomycin daily.

SUMARIO

La Viomicina en la Tuberculosis Experimental

La viomicina inhibió el crecimiento de bacilos tuberculosos virulentos (cepa H37Rv) a concentraciones de 5 γ por cc., mostrándose igualmente activa contra

los microbios resistentes y los sensibles a la estreptomicina. Su efecto inhibidor no fué afectado por la presencia de suero.

Una cepa de bacilos tuberculosos sumamente resistente a la acción de la viomicina *in vitro* fué producida tras 8 pases for un medio líquido que contenía concentraciones cada vez mayores de la droga.

La viomicina se mostró sumamente activa para retardar el avance de la tuberculosis experimental en los cobayos, siendo igualmente eficaz contra la enfermedad producida por bacilos tuberculosos ya resistentes o sensibles a la estreptomicina.

La administración de 40 mg., una vez diaria, intramuscularmente, produjo en los cobayos un efecto beneficioso aproximadamente igual al obtenido por 10 mg. diarios de estreptomicina por vía intramuscular.

RESUME

La Viomycine en tuberculose expérimentale

La viomycine inhibe le développement du bacille tuberculeux virulent (souche H37Rv) à la concentration de 5 γ pour cent, son activité est tout aussi accusée contre les microorganismes streptomycino-résistants que contre ceux streptomycino-sensibles. Son action inhibitrice n'a pas été modifiée par la présence de sérum.

Une souche de bacille tuberculeux doué d'une haute résistance à l'action de la viomycine *in vitro* a été obtenue à la suite de 8 transferts en milieu liquide contenant des concentrations de plus en plus fortes de cette substance.

La viomycine s'est montrée très active en retardant les progrès de la tuberculose expérimentale chez le cobaye. Elle a été aussi efficace dans l'infection produite par les bacilles tuberculeux streptomycino-résistants que par ceux streptomycino-sensibles.

L'administration intramusculaire de 40 mg. une fois par jour a donné, chez le cobaye, un résultat approximativement égal à celui obtenu avec 10 mg. par jour de streptomycine intramusculaire.

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THE EFFECT OF VIOMYCIN IN TUBERCULOSIS OF GUINEA PIGS, INCLUDING IN VITRO EFFECTS AGAINST TUBERCLE BACILLI RESISTANT TO CERTAIN DRUGS

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INTRODUCTION

In this report[2] we present an account of preliminary studies which show that viomycin[3] (1, 2) had a marked therapeutic effect on tuberculosis in guinea pigs in which treatment was not started until the disease had progressed extensively. Data are also presented showing that viomycin inhibits the growth *in vitro* of tubercle bacilli which are resistant to streptomycin, to para-aminosalicylic acid, and to neomycin.

Methods

Each of 26 guinea pigs weighing approximately 850 gm. each was inoculated subcutaneously over the sternum with 0.1 mg. (moist weight) of virulent human-type tubercle bacilli (H37Rv). Twenty-seven days later, 6 animals were killed and were found to have grossly visible lesions of tuberculosis in the usual organs of predilection, as shown schematically in figure 1. The remaining 20 animals were then divided into three groups consisting, respectively, of 8 untreated animals, 6 animals to be treated with 6 mg. of streptomycin once daily, and 6 animals to be treated with 12.5 mg. of viomycin twice daily. The drugs were given in 1 ml. of solution injected into the axillary space.

The daily dose of 6 mg. of streptomycin was selected for comparison with the results obtained with viomycin because that amount of streptomycin has been shown to cause a marked regression and healing of tuberculous disease in guinea pigs (3). The dose of viomycin, 12.5 mg. twice daily, was based on the amount of material available when the experiment was started. After two weeks of treatment it was found that this dosage schedule of viomycin was being tolerated as determined by the maintenance of body weight, and it was, therefore, continued for the rest of the treatment period. All animals were weighed twice weekly during the period when the drug was given.

After sixty-two days of treatment all of the animals were killed. A record was made of the extent of tuberculous lesions visible at necropsy, as shown in figure 1. Tissues were preserved for histopathologic studies.

RESULTS

Drug tolerance: The record of the gain or loss in weight for each group of animals showed that the untreated animals gained an average of 50 gm. each during

[1] From the Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota.

[2] Since preparation of this report, Patelski, Steenken and Wolinsky, and Hobby have published in the *Transactions of the Ninth Streptomycin Conference*, April 1950, their observations that viomycin is active against streptomycin-sensitive and streptomycin-resistant tubercle bacilli *in vitro* as well as in experimental tuberculosis of guinea pigs and mice.

[3] The viomycin was furnished through the courtesy of Parke, Davis & Company, Detroit, Michigan.

the sixty-two days. The animals given 6 mg. of streptomycin once daily gained an average of 100 gm. The group treated with 12.5 mg. of viomycin twice daily had an initial average loss of weight of 20 gm. during the first week which was regained by the second week. There was an average gain in weight of only 15 gm. for each animal in this group by the end of the treatment period. It is not known whether the extra daily injection had an adverse effect on the eating habits

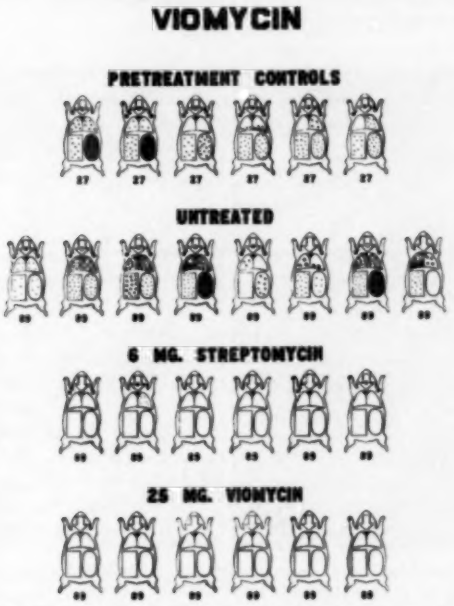
VIOMYCIN
PRETREATMENT CONTROLS
27 27 27 27 27 27
UNTREATED
28 28 28 28 28 28 28 28
6 MG. STREPTOMYCIN
28 28 28 28 28 28
25 MG. VIOMYCIN
28 28 28 28 28 28

FIG. 1. The extent of tuberculous lesions seen at necropsy. The rectangle and oval represent liver and spleen, respectively. The small and large dots represent miliary and nodular lesions, respectively. Diffuse involvement is represented by solid areas. The circle or dot in the arrow indicates an ulcer or an abscess at the site of inoculation. The numerals indicate the number of days after infection when the animals were killed. Treatment was started on the twenty-seventh day after infection and continued for sixty-two days. The dose of streptomycin was given once daily. The viomycin was given in two daily doses of 12.5 mg. each.

of the guinea pigs given viomycin or whether the failure to gain weight was due to some effect of viomycin itself.

Necropsy findings: The extensive involvement seen in the pretreatment control animals killed after twenty-seven days of infection (figure 1) provided evidence of the virulence of the microorganism used for infection. It is presumed that a comparable dissemination of the disease existed in the other animals when treatment was started. In contrast to the extensive involvement of the lungs, liver, and spleen in the pretreatment controls and in the untreated guinea

pigs, there was no grossly visible evidence of disease in these organs in the animals treated with viomycin. There was a striking lack of lesions at the site of inoculation and in the axillary lymph nodes. Four animals, however, had enlarged tracheobronchial lymph nodes. One animal treated with streptomycin had a few miliary lesions in one lung which subsequent microscopic examination showed to be small healing foci. Four guinea pigs in this group had visible lesions at the site of inoculation or the axillary lymph nodes, and 4 had enlarged tracheobronchial lymph nodes.

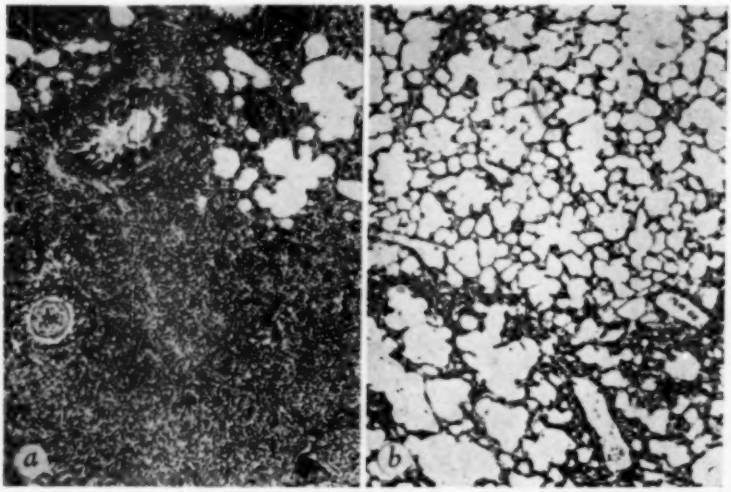

FIG. 2. (a) Section of lung from an untreated guinea pig killed eighty-nine days after infection with 0.1 mg. of virulent tubercle bacilli. There is disseminating involvement of the pulmonary tissue with no evidence of healing (×60). (b) Section of lung from a guinea pig treated with 12.5 mg. of viomycin twice daily for sixty-two days starting twenty-seven days after infection with 0.1 mg. of virulent tubercle bacilli. There are no active tuberculous foci (×60).

Histopathologic studies: The impressive therapeutic effect of viomycin as observed grossly was confirmed by the histopathologic examination. In contrast to the progressive lesions with extensive destruction of tissue seen in the untreated animals, there was little microscopic evidence of tuberculosis in the lungs, liver, and spleen of the animals treated with viomycin. In this group the only evidences of previous disease in these tissues were small fibrotic or calcified foci. The same was true of the animals treated with streptomycin. In both groups of treated animals, however, each animal was found to have small residual areas of necrosis in the tracheobronchial lymph nodes including those which appeared to be normal at necropsy. Figure 2a is representative of the destructive tuberculous process seen in the lungs of the untreated animals killed at the end of the experiment. This type of lesion was also observed in each of the pretreatment

control animals, although to a lesser extent. Figure 2b is typical of the appearance of lungs of the animals treated with viomycin which revealed no tuberculous lesions.

An average index of infection based on the microscopic appearance of the spleen, lungs, liver, and site of inoculation is presented in table 1 (4). Values ranging from 10 to 35, depending on the extent of involvement, were given to each organ with microscopic evidence of active tuberculosis. Values of 1 to 3 were given when there were only healed lesions with fibrosis or calcification. It may be seen from the data in table 1 that the low average index of infection recorded for the spleens, lungs, and livers of the animals treated with viomycin is comparable to that recorded for the animals treated with streptomycin. The presence of active disease at the site of inoculation in 4 of the animals treated

TABLE 1

Numerical Index of the Average Severity of Tuberculosis Based on Histopathologic Characteristics

TREATMENT (DAILY)	NUMBER OF ANIMALS	SPLEEN (MAX.: 35)	LUNGS (MAX.: 30)	LIVER (MAX.: 25)	SITE OF INOCULATION (MAX.: 10)	AVERAGE INDEX OF INFECTION (MAX.: 100)
Pretreatment controls* (not treated)	6	30.0	11.6	13.4	10.0	65.0
Untreated controls	8	24.0	25.0	16.0	10.0	75.0
6 mg. streptomycin once daily†	6	2.0	2.0	1.0	7.0	12.0
12.5 mg. viomycin twice daily†	6	3.0	1.0	1.0	2.0	7.0

* The pretreatment control animals were killed on the twenty-seventh day of infection.

† Treatment started on the twenty-seventh day of infection and continued for sixty-two days.

with streptomycin accounts for the higher average index of infection for this group.

No evidence of toxic effects of viomycin could be detected on microscopic examination of the lungs, livers, spleens, or kidneys of the animals treated with viomycin. There were, however, some small areas of cellular infiltration and fibrosis in the muscle removed from the axillary spaces where the viomycin was administered.

IN VITRO STUDIES

In the experiment described in the preceding paragraphs, which demonstrated the ability of viomycin to cause healing of a progressive tuberculous disease, the infecting microorganism was sensitive to streptomycin, to para-aminosalicylic acid (PAS), and to neomycin. It was of obvious interest to test *in vitro* the effect of viomycin on strains of tubercle bacilli which were resistant to these agents.

In one series of tests viomycin and streptomycin were incorporated in the same medium to determine if one antibiotic would affect the action of the other. Duplicate series of tests,

one in Proskauer and Beck medium (5) and one in egg-yolk agar (6), were done to compare their usefulness in testing the sensitivity of tubercle bacilli to viomycin. Fourfold dilutions of streptomycin and of viomycin were made in each medium, giving concentrations of 128.0, 32.0, 8.0, 2.0, 0.5, and 0.125 γ of antibiotic per ml. of medium, respectively, plus a control tube containing no drug.

In addition to the culture used in the animal experiment, four cultures resistant to streptomycin were tested *in vitro* by the procedure mentioned previously; three cultures resistant to PAS, and one culture resistant to neomycin. The sensitivity tests were read

TABLE 2

The Tuberculostatic Effect of Viomycin in Vitro against Strains of Tubercle Bacilli Resistant to Streptomycin, PAS, and Neomycin*

CULTURE NUMBER	SENSITIVE TO STREPTOMYCIN	RESISTANT TO STREPTOMYCIN	RESISTANT TO PARA-AMINOSALICYLIC ACID			RESISTANT TO NEOMYCIN
	1	2 to 5	6†	7	8	9
Viomycin						
Proskauer & Beck liquid medium	2.0	2.0	2.0	0.5	2.0	2.0
Egg-yolk agar medium	8.0	8.0‡	8.0	8.0	8.0	Not done
Streptomycin						
Proskauer & Beck liquid medium	0.5	128.0	2.0	0.125	0.125	0.125
Egg-yolk agar medium	2.0	128.0	8.0	0.5	0.5	Not done
Streptomycin plus viomycin						
Proskauer & Beck liquid medium	0.5	2.0§	0.5	No growth	0.125	0.125
Egg-yolk agar medium	0.5	8.0	2.0	0.5	0.5	Not done

* Expressed as the maximal amount of the agent which permitted growth after 14 days' incubation at 37°C. Concentrations of viomycin and streptomycin in micrograms per milliliter of medium were 128.0, 32.0, 8.0, 2.0, 0.5, 0.125, and 0.0.

† This culture is resistant *in vitro* to approximately three or four times as much streptomycin as is the usual streptomycin-sensitive strain of tubercle bacilli.

‡ One culture had a few colonies on egg-yolk agar containing 32.0 γ of viomycin per ml. of medium.

§ One culture grew only in the medium containing 0.5 γ of viomycin per ml. of medium.

after fourteen days of incubation, at which time the results were recorded as the maximal concentration of the antibiotic agent which permitted growth.

The results of *in vitro* tests are presented in table 2. It may be seen that all cultures studied, regardless of resistance to the other tuberculostatic agents, were comparable in their sensitivity to viomycin. It is also apparent that the egg-yolk agar medium permitted growth in one concentration higher of viomycin than did the Proskauer and Beck liquid medium, and this also held true for streptomycin. A comparison of the results obtained with streptomycin and those obtained with viomycin suggests that, on a weight basis, the former may be

four times as active *in vitro* against tubercle bacilli as viomycin. When incorporated in the same medium, viomycin and streptomycin appeared to act independently without beneficial or deterrent effect on each other.

COMMENT

A comparison of the therapeutic properties of viomycin and of streptomycin cannot be made from the results obtained in the animal experiment. Although the beneficial effects of the two agents were comparable, the dosage and the frequency of administration were not the same. The data obtained from the *in vitro* studies suggest that, on a weight basis, streptomycin may be about four times as active as viomycin. A measure of the relative therapeutic value of viomycin, however, must await further animal experimentation. Of particular interest was the observation that viomycin is capable of inhibiting the growth *in vitro* of tubercle bacilli resistant to streptomycin and to para-aminosalicylic acid. The occurrence in patients of tubercle bacilli resistant to these two chemotherapeutic agents places a definite limitation on their use. The finding of an antituberculosis agent which may be effective in controlling infections due to tubercle bacilli resistant to streptomycin and para-aminosalicylic acid is of apparent importance. In addition, there is the obvious possibility of using viomycin in combination with streptomycin or para-aminosalicylic acid, or both, not only to obtain any additive therapeutive effect but to determine their combined effect on the occurrence of drug-resistant tubercle bacilli.

SUMMARY

Twenty-six guinea pigs were infected with virulent human-type tubercle bacilli. On the twenty-seventh day of infection 6 were killed and found to have extensive tuberculous involvement of the lungs, liver, and spleen. The remaining animals were divided into three groups consisting, respectively, of 8 untreated control animals, 6 animals treated with 6 mg. of streptomycin daily, and 6 animals treated with 12.5 mg. of viomycin twice daily. After sixty-two days of treatment all animals were killed. Necropsy examination disclosed no tuberculous involvement of the lungs, liver, and spleen of the animals treated with viomycin, and subsequent histologic studies revealed only small healed fibrotic foci, in contrast to the widespread tuberculous disease seen grossly and microscopically in the untreated animals. *In vitro* studies showed that strains of tubercle bacilli resistant to streptomycin, to para-aminosalicylic acid, and to neomycin are equally sensitive to viomycin. When incorporated in the same medium, viomycin and streptomycin appeared to act without mutual benefit or antagonism.

SUMARIO

El Efecto de la Viomicina en la Tuberculosis del Cobayo Comprendiendo los Efectos In Vitro contra los Bacilos Tuberculosos Resistentes a Ciertas Drogas

Veintiséis cobayos fueron infectados con bacilos tuberculosos virulentos de tipo humano. El vigésimoséptimo día de la infección, 6 fueron matados, descu-

briéndose en ellos extensa invasión tuberculosa de los pulmones, hígado y bazo. Los animales restantes fueron divididos en tres grupos compuestos, respectivamente, de 8 animales testigos no tratados, 6 tratados con 6 mg. diarios de estreptomicina y 6 tratados dos veces diarias con 12.5 mg. de viomicina. Al cabo de sesenta y dos días de tratamiento, se mató a todos los animales. El examen autópsico no reveló invasión tuberculosa de los pulmones, hígado o bazo en los animales tratados con viomicina, y los subsiguientes estudios histológicos no mostraron más que pequeños focos fibrosos cicatrizados, lo cual contrastó con la difundida afección tuberculosa observada macro- y microscópicamente en los animales no tratados. Los estudios *in vitro* demostraron que cepas de bacilos tuberculosos resistentes a la estreptomicina, el ácido para-aminosalicílico y la neomicina no dejan de ser sensibles a la viomicina. Incorporadas en el mismo medio, la viomicina y la estreptomicina parecieron actuar sin mutuo beneficio o antagonismo.

RESUME

Action de la Viomycine dans la tuberculose du cobaye, y compris son activité in vitro, contre les bacilles tuberculeux résistant à divers médicaments

Trente-six cobayes ont été inoculés avec des bacilles tuberculeux type-humain. Au vingt-septième jour de l'infection, 6 ont été sacrifiés, ils présentaient des lésions tuberculeuses étendues au niveau des poumons, du foie et de la rate. Les autres animaux ont été répartis en trois groupes comportant, respectivement, 8 animaux témoins non-traités, 6 animaux traités avec 6 mg. de viomycine par jour, et 6 animaux traités avec 12,5 mg. de viomycine deux fois par jour. Tous les animaux ont été sacrifiés au bout de soixante-deux jours de traitement. L'autopsie a montré l'absence de lésions tuberculeuses des poumons, du foie et de la rate chez les animaux traités par la viomycine, des études histologiques ultérieures n'ont révélé que de petits foyers fibreux en voie de cicatrisation, constatation offrant un réel contraste avec les lésions tuberculeuses macroscopiques et microscopiques étendues observées chez l'animal témoin. *In vitro* les recherches ont montré que les souches de bacilles tuberculeux résistant à la streptomycine, à l'acide para-amino-salicylique et à la néomycine sont également sensibles à la viomycine. L'incorporation de la viomycine et de la streptomycine dans un même milieu n'a permis de constater ni un renforcement de leurs effets favorables ni un antagonisme.

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VIOMYCIN[1]

Acute and Chronic Toxicity in Experimental Animals

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Viomycin, an antibiotic isolated from cultures of *Streptomyces puniceus*, has been reported to be active *in vitro* and *in vivo* against both streptomycin-resistant and streptomycin-sensitive strains of tubercle bacilli (1, 2). The object of this report is to present some of the pharmacologic characteristics of this new antibiotic in experimental animals.

Materials

Viomycin is a strong base from which the neutral crystalline sulfate and hydrochloride are readily formed. Both the sulfate and the hydrochloride are quite soluble in water, and are absorbed following parenteral or oral administration.

Crystalline viomycin hydrochloride or viomycin sulfate[2] were used throughout these studies. Dosages are expressed in terms of equivalent weight of pure viomycin base, one microgram of which is defined as one unit.

ACUTE TOXICITY

The acute toxicity of viomycin sulfate was determined in 18- to 22-gram male albino mice of the Rockland Swiss strain. All mice dosed with viomycin sulfate were under constant observation for three to eight hours after injection and daily thereafter up to seven days. The intravenous and subcutaneous LD_{50} (and 19/20 confidence limits) (3) of viomycin sulfate is equivalent to 241 (207–276) and 1,381 (1,278–1,491) mg. of pure base, respectively, per kg. of body weight. The oral toxicity of viomycin sulfate is very low. Oral administration of viomycin sulfate equivalent to 7,500 mg. of pure base per kg. was tolerated by a group of 40 mice.

The symptoms observed in mice following the intravenous injection of a lethal dose of viomycin were those of central nervous system irritation, such as increased irritability, tremors of the extremities, Straub tail reaction, and clonic convulsions, followed by flaccid paralysis. Death, which usually occurred within 5 to 10 minutes, was due to respiratory paralysis. The heart continued to beat for approximately a minute after respirations had stopped.

Daily subcutaneous injections into rats of 50 to 100 mg. of viomycin hydrochloride or sulfate per kg. for a period of five days elicited no toxic symptoms as judged by the general behavior of the animals. No albuminuria developed in these rats.

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[2] All of the viomycin used throughout this study was prepared by the Departments of Biochemical and Chemical Research and Development, Chas. Pfizer & Co., Inc., Brooklyn, New York.

CHRONIC TOXICITY

The effects of daily administration, six days a week, of viomycin sulfate were studied by subcutaneous injections into rats and intramuscular injections into cats and dogs for six weeks or more. Dosages given to individual animals were adjusted at weekly intervals in proportion to weight change. Studies on the chronic toxicity of viomycin in rats, cats, and dogs are still in progress. Further results on these studies, together with gross and histopathologic examinations of tissues and organs of animals sacrificed at the end of the experiments, will be reported at a later date.

Rats: Groups composed of 10 young rats each, weighing 42 to 64 gm., were given daily subcutaneous injections of doses of 50, 100, and 200 mg. of viomycin sulfate per kg. The rats receiving daily injections of 50 and 100 mg. of viomycin

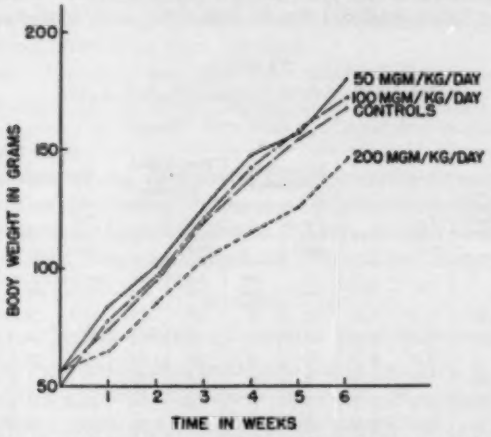

FIG. 1. Growth curves of young rats receiving daily subcutaneous injections of viomycin sulfate.

sulfate per kg. made an average body-weight gain of 235 and 253 per cent, respectively, at the end of six weeks. The control rats had an average weight gain of 213 per cent during the same period. No ill effects were demonstrated in these rats over the six-week period, as judged by the general appearance, behavior, and survival of these rats. There was no significant increase in the albuminuria in the treated rats. The average weight gain of rats receiving daily doses of 200 mg. of viomycin sulfate per kg. was only 172 per cent. Whether the lag in growth of these rats (figure 1) is significant or not has to be judged by observation made over a longer period of time.

Cats: Four cats were given daily intramuscular injections of either 50 or 100 mg. of viomycin sulfate per kg. Each cat was examined daily. The animals receiving 100 mg. of viomycin sulfate per kg. developed changes in gait and posture in twenty-five to thirty-three days. Similar effects were observed in forty

to fifty-three days in the animals receiving daily doses of 50 mg. per kg. The affected cats at first showed some weakness of the hind limbs. Gradually they exhibited an unsteady, weaving gait which at first also resulted from disability limited to both hind limbs and which increased in severity with continued doses of viomycin. These symptoms were accompanied by gradual loss of righting reflex when the cat was dropped in the supine position from a height of one meter, and decrease in nystagmus was evoked by rotation. There was no increased salivation, however, in any of these animals. Studies are in progress to determine whether these symptoms and signs of drug toxicity are reversible if the drug is stopped.

Dogs: In two groups of 3 female dogs, each was given daily intramuscular injections of either 50 or 100 mg. of viomycin sulfate per kg. Frequent observations on these dogs included tests of liver function (bromsulfalein retention), kidney function (phenolsulfonphthalein excretion), and blood counts (table 1).

TABLE 1

Chronic Toxicity of Viomycin Sulfate in Dogs

VIOMYCIN TREATMENT			RESULT									
Dog Number	Dosage	Number of Days on Treatment	Body Weight		Bromsulfalein Retention		Phenolsulfonthalein Excretion		Hemoglobin		Leukocyte Count	
			Pretreatment	Final	Pretreatment	Final	Pretreatment	Final	Pretreatment	Final	Pretreatment	Final
	mg./kg./day		kg.	kg.	per cent	per cent	per cent	per cent	gm. per 100 cc.	gm. per 100 cc.	$\times 10^3$/cu. mm.	$\times 10^3$/cu. mm.
94	50	150	2.8	6.1	0	0	70	70	11.0	13.5	8.8	9.2
99	50	150	5.3	5.8	0	0	68	68	11.4	14.4	16.0	19.0
100	50	7	5.4	5.7	0	0	65	68	15.6	15.2	16.0	15.2
101	100	150	7.2	11.2	0	0	70	68	12.0	14.5	11.2	12.3
102	100	150	5.4	9.1	0	0	70	70	12.6	13.9	12.3	12.9
103	100	150	6.0	7.7	0	0	70	70	12.2	13.6	7.2	8.7

One dog given the lower dosage of viomycin died on the seventh day of treatment. It is very probable that this dog died of intercurrent disease rather than as a result of intoxication with viomycin. The remainder of the dogs tolerated the daily injections well, and continued to be normal up to the time of writing this report (150 days). No significant change was noted in erythrocyte count, total and differential leukocyte counts, or in tests of liver and kidney function. No alteration in behavior referable to drug toxicity was detected in any of these animals during the entire course of observation. The urine of all animals remained free of albumin and reducing sugar.

OTHER PHARMACOLOGIC DATA

Repeated subcutaneous, intramuscular, or intravenous injections of a 10 per cent aqueous solution of viomycin sulfate or hydrochloride caused no signs of local irritation in rats, cats, or dogs. The addition of 0.5 mg. of viomycin per

cc. of Tyrode's solution had no effect on the contractility or rhythmicity of isolated intestines or uteri of rabbits or guinea pigs. Slow intravenous injection (at a rate of 10 mg. per kg. per minute) of 100 mg. of viomycin sulfate per kg. body weight into cats or dogs anesthetized by intraperitoneal Dial caused no appreciable fall in blood pressure or change in respiration.

SUMMARY

Studies of the acute and chronic toxicity of viomycin sulfate indicate that this new antibiotic is of relatively low toxicity. The acute intravenous and subcutaneous LD_{50} of viomycin sulfate in mice were 241 and 1,381 mg. per kg., respectively.

No ill effects were demonstrated in rats given daily subcutaneous injections of 50 and 100 mg. of viomycin per kg. over a period of six weeks. Daily intramuscular injections of 50 and 100 mg. of viomycin sulfate per kg. were tolerated by dogs for a period of more than 150 days.

Disturbances of posture and gait were observed in cats receiving daily intramuscular injections of 100 or 50 mg. of viomycin sulfate per kg. These disturbances appeared in 25 to 33 days with the larger dose and in 34 to 45 days with the smaller dose.

Viomycin is nonirritating. A 10 per cent solution can be given subcutaneously, intramuscularly, or intravenously without causing local pain, necrosis, or thrombophlebitis. Slow intravenous injection of 100 mg. of viomycin per kg. to anesthetized cats or dogs caused no appreciable drop in blood pressure.

SUMARIO

La Viomicina y su Toxicidad Aguda y Crónica en los Animales de Experimentación

El sulfato de viomicina, nuevo antibiótico activo *in vitro* e *in vivo* contra las cepas tanto estreptomicinorresistentes cuanto estreptomicinosensibles de los bacilos tuberculosos, posee una toxicidad relativamente baja.

La DL 50 aguda de sulfato de viomicina por las vías endovenosa y subcutánea en los ratones fué de 241 y 1381 mg. por kg., respectivamente.

No se descubrieron efectos adversos en ratas que recibieron inyecciones subcutáneas diarias de 50 y 100 mg. de viomicina por kg. de peso durante un período de seis semanas.

Inyecciones intramusculares diarias de 50 y 100 mg. de sulfato de viomicina fueron toleradas por grupos de 3 perros para cada dosis durante un período de más de 150 días.

Observáronse trastornos de la postura y de la marcha en gatos que recibieron inyecciones intramusculares diarias de 100 ó 50 mg. de sulfato de viomicina en plazos de 25 a 33 ó 34 a 45 días, respectivamente.

La viomicina no es irritante, pudiendo administrarse una solución al 10 por ciento subcutánea, intramuscular o intravenosamente, sin ocasionar dolor, esfacelo o tromboflebitis locales.

La inyección endovenosa lenta de 100 mg. de viomicina por kg. de peso a los perros o gatos anestesiados no ocasionó baja apreciable de la tensión sanguínea.

RESUME

Toxicité aiguë et chronique de la Viomycine chez l'animal en expérience

Le sulfate de viomycine, nouvel antibiotique actif *in vitro* et *in vivo* contre les souches de bacilles tuberculeux streptomycino-résistantes et contre celles streptomycino-sensibles, a une toxicité relativemente faible.

La dose léthale 50′ de sulfate de viomycine intraveineux et sous-cutané chez la souris a été respectivement de 241 et 1381 mg./kg.

Aucun effet nocif n'a été démontré chez le rat recevant par voie sous-cutanée 50 et 100 mg./kg. de sulfate de viomycine par jour pendant six semaines.

L'injection intramusculaire quotidienne de 50 et 100 mg. de sulfate de viomycine a été bien tolérée par des chiens, répartis en groupe de 3 pour chaque dose, pendant une période de plus de 150 jours.

Chez le chat recevant une injection intramusculaire de sulfate de viomycine à la dose de 100 ou de 50 mg .par jour, des altérations de l'attitude et de la démarche se sont produites, respectivement, au bout de 25 à 33 jours ou de 34 à 45 jours.

La viomycine ne cause pas d'irritation. Une solution à 10 pour cent peut être injectée par voie sous-cutanée, intramusculaire ou intraveineuse sans causer de douleur locale, de nécrose ou de thrombophlébite.

L'injection intraveineuse lente de 100 mg./kg. de viomycine chez le chat ou le chien anesthésié ne détermine aucune chute de tension appréciable.

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THE TOXICITY OF VIOMYCIN IN HUMANS[1, 2]

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INTRODUCTION

The inhibitory action of viomycin on *Mycobacterium tuberculosis in vitro* and its suppressive effect on experimental murine tuberculosis (1, 2) have indicated a potential field of usefulness of this drug in human tuberculosis. The data regarding toxicity of viomycin for mice, rats, and dogs (3) have shown that the drug was of sufficiently low toxicity to permit studies of its administration to humans. Accordingly, a study has been undertaken to determine the effect of the administration of viomycin in humans. The results of this study constitute the basis of the present report.

Materials and Methods

Drug: Viomycin[4] was available in one-gram vials of the highly purified hydrochloride or sulfate salt. The potency of the hydrochloride was the equivalent of 825 γ of pure viomycin base per mg., while the potency of various lots of the sulfate ranged from 755 to 908 γ of pure base per mg. A solution containing 1.0 gm. of viomycin in 4.0 cc. of distilled water was utilized for intramuscular injection. The pH of viomycin sulfate in such a solution was 4.2.

Sensitivity tests: The minimal inhibitory concentrations of viomycin and of streptomycin for the strains of tubercle bacilli isolated from patients in this study were determined by means of a twofold or tenfold serial dilution technique previously described (4) using Tween-albumin medium containing 0.02 per cent Tween 80 and 0.5 per cent bovine albumin. The standard solution of viomycin was prepared by dissolving viomycin sulfate of known potency in distilled water. The potency of the standard was expressed as the equivalent concentration of pure viomycin base. The sensitivity of the organism was expressed as the minimal concentration of drug required to prevent macroscopically discernible growth after incubation for 14 days at 37°C.

Clinical observations: In addition to the initial history and physical examination, each patient was examined by indirect laryngoscopy, and determinations were made of vestibular and of auditory function (4) prior to the institution of viomycin. A chest roentgenogram and electrocardiogram were obtained, and examinations of sputum by direct smear and by culture were made in each patient. The following laboratory examinations were made prior to the administration of viomycin: complete blood count; sedimentation rate; urin-

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[4] Supplied by Charles Pfizer and Co., Brooklyn, N. Y.

alysis; examination of urine for calcium content (Sulkowitch test); chemical determination of the blood urea nitrogen, serum chlorides, carbon dioxide combining power, calcium, phosphorus, sodium, potassium, bilirubin, alkaline phosphatase, thymol turbidity, and total proteins with albumin-globulin ratio, and the bromsulfalein excretion. These procedures were repeated at intervals of a few days to four weeks throughout the period of viomycin administration and for variable periods after the drug had been discontinued. The chemical determinations were made by conventional laboratory methods mentioned in a previous report (5). Serum sodium and potassium concentrations were determined with a flame photometer.

Case Material and Treatment Regimen

Ten patients with pulmonary tuberculosis were treated with viomycin. Listed in table 1 are pertinent data on these patients, including sex, age, race, extent and duration of tuberculosis, complicating illnesses, and previous specific antimicrobial treatment. All patients had far advanced pulmonary disease with cavitation, and all were febrile. Tubercle bacilli were cultured from specimens of sputum in each case prior to therapy. The known duration of tuberculosis had been from two months to eight years, and all but 2 patients had received previous treatment with streptomycin which had been completed one week to one year before the start of viomycin (table 1). In addition, 7 patients had received combined treatment with para-aminosalicylic acid and streptomycin, and 2 patients had received 4-acetylaminobenzaldehyde thiosemicarbazone and terramycin, respectively. Periods of bed rest lasting from one and one-half months to 19 months had immediately preceded the start of viomycin therapy. Three patients had had collapse therapy in the form of pneumothorax or pneumoperitoneum previous to the present study.

The patients were maintained on bed rest during the period of viomycin administration. No collapse therapy or other specific antimicrobial drugs were employed during the study. The dosage of viomycin which was employed and the duration of treatment in these patients have been presented in table 2. The daily dose of viomycin ranged from 30 to 75 mg. per kg., and the period of therapy lasted from 14 to 182 days. The total daily dose was given intramuscularly in two or three equal fractions at 6- or 12-hour intervals respectively.

OBSERVATIONS

Toxic Reactions

Local: The 3 patients who received the hydrochloride salt developed painful, tender, fluctuant, and indurated nodules at the site of injection in the buttocks which necessitated the application of ice packs and the use of anodynes. When the sulfate salt was substituted no further local reactions were encountered.

Hypersensitivity: Two patients developed eosinophilia within the first three weeks of therapy. The percentage of eosinophils in the differential leukocyte count ranged from 10 to 30. Eosinophilia persisted for the duration of therapy and then gradually subsided after the drug was withdrawn. The development of eosinophilia could not be correlated with the administration of any particular

TABLE 1

Clinical Data on Patients Prior to Administration of Viomycin

PATIENT, SEX, AGE, RACE	CLASSIFICATION OF PULMONARY TUBERCULOSIS AND DURATION OF DISEASE	PREVIOUS SPECIFIC ANTIMICROBIAL THERAPY
1. S. R. F 30 W	Unilateral cavitation, contralateral spread, III-C, 2 years; nonulcerative tuberculous laryngitis	SM 1.0 gm/day × 56 days completed 15 months before; PAS 12 gm/day × 230 days completed 2 weeks before
2. G. H. F 24 N	Extensive bilateral cavitary, III-C, 1 year	SM 1.0 gm/day and PAS 12 gm/day × 58 days completed 2 weeks before
3. R. D. M 40 N	Extensive bilateral, very large cavities, III-C; 6 months	SM 1.0 gm/day × 42 days; SM 1.0 gm/day and PAS 12 gm/day × 42 days completed 1 week before
4. G. S. F 27 W	Extensive bilateral cavitary, III-C; 8 years; multiple sclerosis, urinary tract infections	SM 1.0 gm/day × 84 days; PAS 12 gm/day × 63 days; DHSM 5 gm/day × 77 days completed 1 year before; Tbl 200 mg/day × 76 days completed 50 days before
5. A. T. M 30 N	Extensive bilateral cavitary, III-D; 1 year	SM 1.0 gm/day and PAS 12 gm/day × 42 days completed 6 months before; TM 4 gm/day × 42 days completed 1 day before
6. E. R. M 38 N	Extensive bilateral cavitary, III-C; 8 months	SM 1.0 gm/day and PAS 12 gm/day × 21 days completed 40 days before; PAS 12 gm/day × 40 days completed 3 weeks before
7. M. D. M 38 W	Extensive bilateral infiltration, unilateral cavitation, III-C; 8 months; latent syphilis	SM 1.0 gm/day × 42 days completed 18 months before
8. R. O. M 53 W	Extensive bilateral cavitary, III-D; 2 years; chronic otitis media and mastoiditis	SM 1.0 gm/day × 42 days; SM 3 gm/day and PAS 12 gm/day × 16 days completed 4 months before
9. R. R. M 25 N	Extensive unilateral cavitary, III-C; 4 months	None
10. J. O. M 46 W	Extensive bilateral infiltration, unilateral cavitation, III-C; 2 months	None

SM = Streptomycin DHSM = Dihydrostreptomycin PAS = Para-aminosalicylic acid Tbl = Tibione (4-acetylaminobenzaldehyde thiosemicarbazone) TM = Terramycin.

TABLE 2

Dosage of Viomycin and Toxic Reactions Encountered during Its Administration

PATIENT	DURATION OF THERAPY	DOSAGE OF VIOMYCIN			TOXIC REACTIONS		
		Gm/Day	Mg/kg/Day	Total Gm.	Hypersensitivity	Renal	Neurologic
1. S. R.	101 20 — Total 121	2.5 2.0	50 40	252 40 — 292	Eosinophilia after 21 days	Albuminuria and cylindruria after 13 days	Dizziness, weakness, tetany, paresthesias after 120 days
2. G. H.	182	2.0	50	364	Eosinophilia after 9 days; urticaria after 64 days	Albuminuria and cylindruria after 13 days; slight elevation of BUN after third week	None
3. R. S.	71 9 — Total 80	2.5 2.0	50 40	178 18 — 196	None	Albuminuria and cylindruria after 3 days	None
4. G. S.	73	2.0	40	146	None	(Pre-existing urinary tract infection)	Dizziness, weakness, tetany after 64 days
5. A. T.	60	2.0	40	120	None	Albuminuria and cylindruria after 14 days	Weakness, lethargy after 42 days
6. E. R.	68	3.0	55	204	None	Albuminuria and cylindruria after 12 days; slight elevation of BUN after second week	Tinnitus after 53 days; diminished vestibular function after 45 days; 10 to 20 per cent bilateral hearing loss 10 weeks after discontinuance of therapy

TABLE 2—*Continued*

PATIENT	DURATION OF THERAPY	DOSAGE OF VIOMYCIN			TOXIC REACTIONS		
		Gm/Day	Mg/kg/Day	Total Gm	Hypersensitivity	Renal	Neurologic
7. M. D.	16 74 — Total 90	2.0 3.0	50 75	32 222 — 254	None	Albuminuria and cylindruria after 12 days; slight elevation of BUN after fourth week	Tinnitus, headache, after 50 days; diminished vestibular function and 20 to 30 per cent bilateral hearing loss after 86 days
8. R. O.	16 32 — Total 48	1.0 2.0	30 65	16 64 — 80	None	Albuminuria and cylindruria after 11 days; slight elevation of BUN after third week	Dizziness, weakness after 31 days; diminished vestibular function after 15 days
9. R. R.	4 51 12 — Total 67	4.0 2.0 3.0	70 35 50	16 102 36 — 154	None	Albuminuria and cylindruria after 13 days	Nausea and vomiting after second day; disappeared when dose was reduced
10. J. O.	4 10 — Total 14	2.0 3.0	30 45	8 30 — 38	Erythematous rash after 4 days; necessitated discontinuance of therapy	Albuminuria and cylindruria after first day	None

N.B: R. S., G. S., A. T., E. R., and R. O. developed changes in serum electrolytes which included hypokalemia, hyocalcemia, hypophosphatemia, hypochloremia, and alkalosis.

lot of viomycin. One of these 2 patients (G. H.) developed a pruritic, urticarial rash during the tenth week of therapy which subsided despite continuance of viomycin. Another patient (J. O.) developed a generalized, erythematous pruritic

rash commencing on the fourth day of therapy which persisted and necessitated discontinuance of viomycin after two weeks.

Renal: Nine patients developed albuminuria, from a faint trace to 3 plus, within two weeks of the start of viomycin. This was accompanied by the appearance of variable numbers of hyaline and granular casts, erythrocytes and leukocytes in the centrifuged urine sediment on microscopic examination. The tenth patient had a concurrent chronic urinary tract infection and had abnormal urine at the time viomycin was begun. The blood urea nitrogen was elevated after the second week of therapy in 4 patients and returned to normal within seven to ten days after the drug was discontinued. The highest concentration noted was 35 mg. per 100 ml. Fluid intake and output were satisfactorily maintained in all patients throughout the study period.

Electrolyte imbalance: Five patients complained of varying degrees of anorexia, nausea, weakness, lassitude, muscle cramps, and paresthesias after four to six weeks of continuous treatment with viomycin. Two of these patients (G. S. and S. R.) exhibited overt tetany after 40 and 120 days of therapy, respectively. Four patients exhibited electrocardiographic changes consistent with electrolyte abnormalities. These electrocardiographic changes included low amplitude of the QRS complexes, depressed RT segments, and low amplitude of the T waves.

Striking and unusual alterations in the concentrations of the serum electrolytes were noted in these patients. The abnormalities consisted of a fall in concentration of the serum potassium, calcium, phosphorus, and chlorides, and an increase in the carbon dioxide combining power of the serum. These alterations of the serum electrolytes were not discovered until late in the course of therapy in one patient. The data for the remaining patients have been grouped in table 3. From this table it may be noted that the first deviations from normal which were observed were a diminution in the concentration of the serum potassium and chlorides and an increase in the serum bicarbonate concentration. These changes became evident within the first four weeks of therapy. In spite of the chemical findings of alkalosis in the blood, the pH of the urine remained below 7.0 except in the 2 patients who developed the most severe alkalosis and in whom the urine was highly alkaline. There continued to be excessive amounts of calcium in the urine, as indicated by the Sulkowitch reaction, even when the serum calcium was as low as 6.5 mg. per 100 ml.

Intensive therapy with potassium and calcium salts was required to correct the electrolyte abnormalities and to reverse the clinical symptoms and electrocardiographic changes. In 4 patients it became necessary to discontinue viomycin after 121, 73, 60, and 48 days, respectively, because of electrolyte changes. The fifth patient was able to continue to take viomycin while receiving adjuvant therapy with potassium chloride, 1.0 gm. per day orally.

Neurologic: In addition to the neurologic changes which were associated with the electrolyte abnormalities, 2 patients complained of low-pitched tinnitus which appeared after 50 and 53 days, respectively, and persisted for several weeks after the drug was discontinued. Two patients had diminished vestibular function as measured by response to caloric stimulation after 45 and 86 days of drug therapy.

TABLE 3

Concentration of Serum Electrolytes in Four Patients Showing Abnormal Values While Receiving Viomycin

PATIENT	DURATION OF THERAPY DAYS	ELECTROLYTE mEq/L OR mMOLS/L	WEEKS AFTER START OF THERAPY												
			0	1	2	3	4	5	6	7	8	9	10	11	12
G. S.	73	Ca	4.5						3.4	3.7	4.0	3.8	3.2	4.2	4.6
		P	1.6						2.6	2.5	2.3	2.5	[illegible]	2.1	1.9
		K							3.6		2.0	3.5	2.9		4.2
		Na							136		133	107	127		136
		Cl								94	92		88	90	96
		HCO_3								30	28		32	30	30
A. T.	60	Ca							4.5	4.3	3.9	3.2	3.2	4.5	
		P	2.8						2.5	1.6	2.0		2.0	2.4	
		K							3.6	4.1	3.3	3.5		5.0	
		Na							127	136	128	130		137	
		Cl							77	80	76		83	86	
		HCO_3							36	32	32		32	31	
E. R.	68	Ca	4.8	4.7	5.0	5.1	4.8	4.8	4.4	5.4	4.8	4.8	4.8		
		P	2.5	3.5	2.8	2.3	2.1	2.0	1.7	1.9	2.1	2.1	1.8		
		K	4.9	4.5	4.6	4.0	3.6	3.6	4.2	3.3	3.5	3.8	3.8		
		Na	140	136	132	141	130	139	136	130	129	135	137		
		Cl	99	99		96		94		90		92	96		
		HCO_3	28	30		34		30		30		28	30		
R. O.	48	Ca	4.2	4.3	4.2	4.2	3.8	3.6	4.0	4.0	3.6	3.6			
		P	2.1	2.3	2.1	2.0	1.3		1.3	1.7	1.2	1.2			
		K	5.2		4.1	3.9	3.0	3.8	4.2	3.1	2.5	3.9			
		Na	135		132	136	130	132	137	135	122	136			
		Cl	100	95		93	88		88		80		93		
		HCO_3	27	32		32	34		30		38		31		

Normal values: Ca = 4.5 to 5.2 mEq/L K = 3.8 to 5.1 mEq/L Cl = 98 to 101 mEq/L
P = 1.8 to 2.6 mEq/L Na = 130 to 148 mEq/L HCO_3 = 22 to 27 mMols/L

These 2 patients sustained degrees of hearing loss which ranged from 10 to 20 per cent and from 20 to 30 per cent, respectively. The hearing loss was noted in one of these patients after 86 days of therapy, and it did not become apparent in the other patient until 10 weeks after viomycin had been withdrawn. Another

TABLE 4

Sensitivity of M. Tuberculosis to Viomycin and Streptomycin during Treatment with Viomycin

PATIENT	DURATION OF THERAPY DAYS	TIME OF CULTURE AFTER START OF THERAPY DAYS	SENSITIVITY (γ/cc.)*	
			Viomycin	Streptomycin
1. S. R.	121	Before	6.2	>1,000
		74	50	
		120	50	
		147	50	
2. G. H.	182	Before	6.2	<1
		61	12.5	
		80	25	
		94	25	<1
3. R. S.	80	Before	6.2	>1-<10
		39	6.2	
		62	100	
		109	100	>1-<10
4. G. S.	73	Before	12.5	>1,000
		46	12.5	
		87	100	
5. A. T.	60	Before	12.5	>1,000
		20	12.5	
		72	50	
6. E. R.	68	Before	6.2	<1
		19	12.5	
		40	12.5	<1
7. M. D.	90	Before	12.5	<1
		19	12.5	
		36	12.5	
8. R. O.	48	Before	6.2	
		22	12.5	>1-<10

* 14-day readings.

patient had concomitant middle-ear disease with partial deafness and loss of vestibular response to caloric stimulation after 15 days of treatment. Each of these patients had received streptomycin prior to the administration of viomycin (table 1).

A summary of the toxic reactions encountered in the study has been given

in table 2. No adverse effects on the hematopoietic system or liver which could be related to viomycin were noted.

Clinical Course Under Treatment

Two patients exhibited good-to-moderate, three slight, and three no improvement in the status of their pulmonary tuberculosis while receiving treatment with viomycin. Two patients continued to have progression of disease as evidenced by study of the serial chest roentgenograms. In two patients (M. D. and G. H.) the sputum cultures for tubercle bacilli became negative after 45 and 120 days of treatment, respectively. These patients also had the most favorable clinical response with regard to fall in temperature, diminution in volume of sputum, improvement in the appearance of the chest roentgenogram, and gain of weight. Both patients had previously received streptomycin.

Bacterial Sensitivities

In table 4 are recorded the viomycin and streptomycin sensitivities of the infecting strains of *M. tuberculosis* isolated from the patients in this study. The initial sensitivities to viomycin were 6.2 to 12.5 γ per cc. Increases in resistance to viomycin of two- to sixteen-fold were noted during therapy. The higher degrees of resistance, i.e., eight- to sixteen-fold, were not observed before completion of 62 days of treatment. It is also apparent from table 4 that organisms highly resistant to streptomycin were initially sensitive to viomycin. Similarly, organisms that became resistant to viomycin, i.e., requiring 100 γ per cc. for inhibition, did not lose their sensitivity to streptomycin.

DISCUSSION

Despite the occurrence of certain toxic reactions, it is apparent from this study that humans are able to tolerate viomycin in a dosage of 50 to 75 mg. per kg. per day intramuscularly for long periods of time. The signs of renal toxicity and the alterations of the serum electrolytes observed during the period of viomycin administration proved to be reversible when administration of the drug was discontinued. In some instances it was found possible to continue viomycin for various intervals after the appearance of abnormalities in the serum electrolytes provided adjuvant therapy with mineral salts was given concurrently.

The problem of determining the cause of the disturbances in the serum electrolytes encountered in these patients was complicated by many variables other than the drug. All of the patients were severely ill and had anorexia, difficulty in maintaining adequate nutrition, and some degree of bodily wasting. In addition, several patients had had episodes of vomiting before viomycin therapy and similar episodes occurred occasionally during therapy. Although it was not clearly attributable to viomycin administration, the vomiting may have contributed to the erythrocyte imbalance. Those with the most extensive pulmonary disease probably were having some degree of impairment of respiratory exchange of carbon dioxide leading to respiratory acidosis (6).

The diminished values of calcium, phosphorus, chlorides, and potassium in the

serum may be explained by several possible mechanisms. Prominent among these are diminished intake or absorption of these substances from the intestines, increase excretion by the kidneys or other routes, and shift of these ions into the intracellular fluid compartment or, in the case of calcium and phosphorus, into the bones. While the data are insufficient to establish the exact nature of the processes in operation, the evidence of irritation of the renal tubules, the persistence of a positive Sulkowitch reaction in the presence of hypocalcemia, and the slight elevation of the blood urea nitrogen suggest that the major cause may have been a toxic action of viomycin upon the kidney, resulting in increased loss of electrolytes in the urine.

Manifestations of neurotoxicity were inconstant and their interpretation complicated by the fact that all patients had previously received streptomycin. Nevertheless, the occurrence in 2 patients of definite impairment of vestibular function and partial deafness while receiving viomycin indicates that patients receiving this drug in the future must be observed for signs of vestibular dysfunction and deafness.

It was not the chief purpose of the present study to define the therapeutic capabilities of viomycin, nor would this be possible from such a small number of cases. In all but 2 instances, the individual patients were first selected because they had failed to respond favorably to antimicrobial therapy with streptomycin and PAS and were unsuitable for collapse or surgical therapy. Thus, failure to demonstrate in these patients any conspicuous improvement on viomycin therapy does not preclude the possibility that this agent may have therapeutic activity approaching or even equaling that of streptomycin. There is little in the present experience, however, to indicate that viomycin actually does have such therapeutic activity. The final assessment in each of those few patients whose condition improved has not, in our judgment, permitted a definite attribution of therapeutic value to the drug, since the improvement was slow and only of slight to moderate degree. A selection of cases more favorable for therapeutic evaluation, as well as treatment of a larger series, will be necessary to determine the clinical value of this drug. The emergence of bacterial resistance was suggestive of antibacterial activity of viomycin within the human host.

The emergence of strains of *M. tuberculosis* resistant to viomycin followed the same general pattern as that for streptomycin and dihydrostreptomycin (7, 8). Bacterial resistance to viomycin, however, was of lower degree than that encountered in the use of streptomycin. This is in accord with the results of *in vitro* experiments of Hobby and others (2).

The limitations and disadvantages of streptomycin therapy in tuberculosis have now been defined, and clearly point to the need for additional antituberculous drugs which can be used in lieu of, together with, or after streptomycin or dihydrostreptomycin. From the nature of the toxic reactions, it is not likely that viomycin and streptomycin should be administered concurrently. Moreover, the degree of toxicity of viomycin observed in the present study would not be acceptable in a drug of only low antituberculous activity. If the antituberculous activity of viomycin proves to be high, however, the types and degree of toxicity noted

would be tolerable. Thus, the results of the present study, although indicating clearly the need for careful control of administration of this drug, would seem to warrant the continued investigation of this compound in human tuberculosis.

SUMMARY

Ten patients with far advanced pulmonary tuberculosis have been treated with viomycin in daily doses of 30 to 75 mg. per kg. by intramuscular administration for periods of 14 to 182 days. Evidences of toxicity have included renal irritation in all patients, abnormalities of the serum electrolytes in 5 patients, slight elevation of the blood urea nitrogen in 4 patients, impairment of vestibular function and partial deafness in 2 patients, and minor hypersensitivity reactions in 3 patients.

The abnormalities observed in the serum electrolytes included hypokalemia hypocalcemia, hypophosphatemia, hypochloremia, and increase in carbon dioxide combining power. The mechanism of development of these abnormalities has not been clarified and does not seem entirely explained by a toxic action of viomycin on the kidney. The abnormalities necessitated discontinuance of the drug in 4 patients and intensive replacement therapy with potassium and calcium salts. It is suggested that the concurrent administration of these adjuvants with viomycin in the future may serve to diminish or possibly prevent development of electrolyte imbalance.

The strains of *M. tuberculosis* isolated from the patients in this study were sensitive before treatment to 6.2 to 12.5 γ per ml. of viomycin *in vitro*. An eight- to sixteen-fold increase in resistance to viomycin was found to occur after 60 to 70 days of treatment, and this emergence of resistance followed the same general pattern as the development of bacterial resistance to streptomycin. Bacterial sensitivity or resistance to viomycin was independent of that to streptomycin.

In this preliminary study of viomycin in human tuberculosis the selection of case material was such as to preclude a definitive therapeutic evaluation. Some promise of favorable effect upon the disease may be inferred from the occurrence of slight-to-moderate improvement in a few instances. The toxicity encountered was not of sufficiently serious degree or character to prevent the eventual use of the drug provided it can be shown to possess substantial antituberculous activity. Consequently, further clinical trials seem warranted in patients more suitable for therapeutic evaluation than would have been justified in the present exploratory study.

SUMARIO

La Toxicidad de la Viomicina en los Seres Humanos

Diez enfermos con tuberculosis pulmonar muy avanzada fueron tratados con viomicina a dosis diarias de 30 a 75 mg. por kg. de peso por vía intramuscular durante períodos de 14 a 182 días. Los signos de toxicidad comprendieron irritación renal en todos los sujetos, anomalías de los electrolitos del suero en 5, leve elevación del nitrógeno de urea sanguíneo en 4, disfunción vestibular y sordera parcial en 2 y ligeras reacciones de hipersensibilidad en 3 enfermos.

Las anomalías observadas en los electrolitos séricos abarcaron hipopotasemia, hipocalcemia, hipofosfatemia, hipocloremia y aumento de la facultad para combinar el bióxido de carbono. No se ha esclarecido aun el mecanismo de esas anomalías, ni tampoco parece explicarlo bien ninguna acción tóxica de la viomicina sobre el riñón. Dichas anomalías impusieron el abandono de la droga en 4 enfermos e intensa sucedoterapia con sales de potasio y de calcio. La administración concurrente de dichos coadyuvantes con la viomicina en el futuro quizás sirva para atenuar o posiblemente impedir la aparición de desequilibrio electrolítico.

Las cepas de *M. tuberculosis* aisladas de los pacientes comprendidos en este estudio eran sensibles antes del tratamiento a 6.2 a 12.5 γ por ml. de viomicina *in vitro*. Al cabo de 60 a 70 días de tratamiento, observóse una resistencia de ocho a dieciséis veces mayor a la viomicina, conformándose esta aparición de resistencia a los mismos patrones generales que la formación de resistencia bacteriana a la estreptomicina. La sensibilidad o resistencia bacteriana a la viomicina fué independiente de la observada con respecto a la estreptomicina.

En este estudio preliminar de la viomicina en la tuberculosis humana, fué tal la selección de casos que impedía una valuación terapéutica definitiva. Cabe inferir alguna esperanza de efecto favorable por la ocurrencia de mejoría leve a moderada en algunos casos. La toxicidad notada no fué suficientemente grave en su intensidad o naturaleza para prohibir nuevas pruebas clínicas, las cuales parecen justificadas en enfermos más apropiados para una justipreciación terapéutica que no hubiera sido procedente en el actual estudio explorador.

RESUME

Toxicité de la Viomycine chez l'homme

Dix malades atteints de tuberculose pulmonaire avancée ont été traités par la viomycine à la dose quotidienne de 30 à 75 mg./kg. par voie intramusculaire, pendant une durée de 14 à 182 jours. Les manifestations de toxicité observées one consisté en une réaction inflammatoire rénale chez tous les malades; des anomalies dans la concentration des électrolytes du plasma chez cinq malades; des altérations de la fonction vestibulaire et une surdité partielle chez deux malades; des réaction allergiques légères chez trois malades.

Les anomalies concernant les électrolytes du plasma ont été l'hypopotassémie, l'hypocalcémie, l'hypophosphatémie, l'hypochlorémie et une augmentation du pouvoir de combinaison du CO2. Le mécanisme du développement de ces anomalies n'a pas été éclairci; il ne semble pas facilement explicable par une action toxique quelconque de la viomycine sur le rein. Ces manifestations ont nécessité la suspension de la drogue chez quatre malades et une thérapeutique intensive de remplacement, à l'aide de sels de potassium et de calcium. On prévoit que, dans l'avenir, l'administration concommittante de ces adjuvants et de la viomycine permettra de diminuer, ou même de prévenir le développement du déséquilibre électrolytique.

Les souches de *M. tuberculosis* prélevées sur les malades de cette étude s'étaient montrées, avant tout traitement, sensibles *in vitro* a l'action de la viomycine à la

dose de 6, 2 à 12, 5 γ par ml. Après 60 à 70 jours de traitement on a observé. une augmentation dé la résistance à la viomycine huit à sieze fois plus grande; l'apparition de cette résistance suivait la même évolution que le développement de la résistance bactérienne à la streptomycine. La sensibilité ou la résistance bactérienne à la viomycine était sans relation avec celle existant pour la streptomycine.

Dans cette étude préliminaire de la viomycine en tuberculose humaine, la sélection des cas en faisant l'objet ne permet pas d'apprécier de façon définitive sa valeur thérapeutique. L'espoir d'une action favorable sur la maladie peut être. déduit de l'apparition d'une amélioration lègére ou modérée dans quelques cas. La toxicité n'a pas été d'un degré ou d'un caractère suffisamment grave pour prévenir d'autres essais cliniques, et ces assais paraissent justifiés chez des patients, se prêtant mieux à l'appréciation thérapeutique, que ne l'ont permis les cas observés dans l'étude exploratrice actuelle.

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TEN YEARS OF PNEUMOPERITONEUM[1,2,3,4]

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INTRODUCTION

This report presents the results obtained in the treatment of pulmonary tuberculosis with pneumoperitoneum in the ten-year period from 1937 to 1947. Although more than 400 patients received pneumoperitoneum therapy during this period, the discussion will be limited to 246 who had this treatment for at least six months and in whom the average period of observation was twenty-one months.

Composition of Clinical Series

Of the total of 246 patients included in the study, all but 4 had far advanced pulmonary tuberculosis, and these 4 had moderately advanced disease. In the majority of patients, the prognosis was only fair with conventional forms of treatment. In approximately 80 per cent of the patients, a trial of pneumothorax had been made, with resultant failure due either to lack of free pleural space, to inadequate collapse, or to the development of pleural effusion. In 5 per cent, unilateral pneumothorax was maintained during pneumoperitoneum therapy. Eighty per cent of the patients also had temporary phrenic nerve paralysis, with an average of 5.0 cm. elevation of the hemidiaphragm.

In table 1 is presented the age distribution of the patients reported. All patients were over the age of twenty, and approximately two-thirds were between the ages of twenty and thirty-nine years. Only 2 of them were females. Thirteen of the patients were Mexicans and 8, Negroes.

The distribution of cases classified by the duration and type of disease is recorded in tables 2 and 3. For the sake of uniformity, it was decided to rely for this classification on roentogenographic and bacteriologic data rather than on the history. The majority of patients had their disease from one to four years. In more than half, the disease was classified as fibroid, and in only 7 per cent was predominantly exudative disease present.

Roentgenographically demonstrable pulmonary cavitation was present in 207 patients (84 per cent). In 60 per cent, cavitation was confined to one lung. In 12 per cent, the cavitation was in the lower third of the lung. As may be seen in table 4, cavities with a diameter exceeding 5 cm. were present in 56 per cent of the patients.

Cavity closure was considered to have occurred when a previously demonstrable cavity could not be visualized upon roentgenographic study by examination of standard postero-anterior chest films, as well as of special views including laterals, apicograms, and planigrams.

[1] From the Veterans Administration Hospital, San Fernando, California.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

[3] Read before the California Chapter, American College of Chest Physicians, at San Diego, California, April 29, 1950.

[4] This work was aided by a grant from the California Trudeau Society.

Sputum conversion was said to have occurred if adequate amounts, when available, were negative both on microscopic examination of the concentrated specimen and on

TABLE 1

248 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Age Distribution

AGE GROUPS	NUMBER OF PATIENTS	PERCENTAGE OF TOTAL NUMBER OF PATIENTS
20 to 29 years	96	39
30 to 39 years	69	28
40 to 49 years	64	26
50 + years	17	7

TABLE 2

248 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Duration of Disease

DURATION	NUMBER OF PATIENTS	PERCENTAGE OF TOTAL NUMBER OF PATIENTS
Under 1 year	13	5
1 to 4 years	155	63
5 to 10 years	68	28
10 + years	10	4

TABLE 3

248 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Pathological Characteristics

CLASSIFIED AS TO PREDOMINANT TYPE OF DISEASE	NUMBER OF PATIENTS	PERCENTAGE OF TOTAL NUMBER OF PATIENTS
Exudative	16	7
Fibroid	136	55
Mixed	94	38

TABLE 4

248 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Classified as to Total Size of Cavities

SIZE	NUMBER OF PATIENTS	PERCENTAGE OF TOTAL NUMBER OF PATIENTS
Less than 2 cm	13	6
2 to 4 cm	76	37
5 to 10 cm	115	56
10 + cm	3	1

culture. If specimens of sputum were unsatisfactory, the results of cultures of gastric aspirates were then used. The criteria used for "arrest" and "apparent arrest," in addition to those required by the National Tuberculosis Association classification, included nega-

tive cultures of specimens of either sputum or gastric aspirates. In table 5, both "arrested" and "apparently arrested" have been placed in a single category labeled "control of disease."

TABLE 5

246 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Results of Treatment Classified as to Type of Disease

	EXUDATIVE 16 (16)*		FIBROTIC 94 (74)*		MIXED 136 (117)*	
	Number	Per cent	Number	Per cent	Number	Per cent
Cavity closure	13	81	7	9	22	18
Sputum conversion	12	75	5	5	17	12
Control of disease	12	75	5	5	14	10

* First figure represents total number of patients, and figure in parenthesis indicates number with cavitary lesions.

TABLE 6

246 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum
Results of Treatment Classified by Duration of Disease

	UNDER 1 YEAR 13 (13)*		1 TO 2 YEARS 54 (34)*		3 TO 4 YEARS 101 (82)*		5 TO 10 YEARS 68 (68)*		OVER 10 YEARS 10 (10)*	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Cavity closure	11	85	20	59	11	13	0	0	0	0
Sputum conversion	10	70	16	41	8	10	0	0	0	0
Control of disease	10	70	14	32	6	7	0	0	0	0

* First figure represents total number of patients, and figure in parenthesis indicates number with cavitary lesions.

TABLE 7

246 Patients with Pulmonary Tuberculosis Treated with Pneumoperitoneum

	INCREASED	DECREASED	UNCHANGED
Objective signs			
Sputum volume		35%	65%
Weight	10%	5%	85%
Temperature		Generally unaffected	
Subjective symptoms			
Cough		30%	70%
Dyspnea	3%	7%	90%
Appetite	20%	6%	74%

RESULTS

In the entire series, cavity closure occurred in 42 of 207 patients (20 per cent). Conversion of sputum took place in 16 per cent of all patients, with 14 per cent in the cavitary cases. Without further analysis, the therapeutic value of pneumo-

peritoneum would indeed appear very low. However, if one evaluates the effect of treatment as related to type and duration of disease, it is found that the results are significantly influenced by these factors.

The patients classified as having exudative disease, all with pulmonary cavitation, comprise a small part of the total number of cases (table 5). It is significant, however, that in this group cavity closure occurred in 81 per cent of the cases, and sputum conversion in 75 per cent (table 6). Moreover, in this group, 75 per cent of the patients reached either the arrested or apparently arrested stage. In contrast, the group classified as having fibrotic disease showed only insignificant numbers with cavity closure, sputum conversion, or control of their disease. In the mixed group (fibro-exudative), although better than the group with fibrotic disease, more than 80 per cent of the patients failed to achieve control of their disease.

There appears to be a definite parallel between duration of disease and achievement of cavity closure, sputum conversion, and control of the disease. The percentage of patients in whom satisfactory results are obtained shows a precipitous drop in those who have had their disease longer than two years, approaching zero in those whose disease is more than five years old.

OTHER OBSERVATIONS

Approximately one-third of the patients had decrease in both cough and sputum volume. Weight decreased slightly after start of treatment, but otherwise was not significantly influenced. Only a small number of patients had fever of 100°F. or over prior to the institution of pneumoperitoneum. Although more than 80 per cent of the patients had paralysis of the hemidiaphragm combined with pneumoperitoneum, the degree of dyspnea, when present, remained relatively unchanged. Despite the small number who gained weight, twice as many patients stated their appetites had definitely improved.

Complications were few. Air embolism occurred in 2 patients, severe subcutaneous emphysema in one, and peritoneal effusion in 3 patients. Although approximately 15 per cent of the patients complained of abdominal discomfort, in only 3 per cent was the procedure discontinued because of this complaint.

DISCUSSION

No therapeutic measure in tuberculosis is more debatable at present than pneumoperitoneum. Reports continue to appear describing the favorable influence of pneumoperitoneum with phrenic nerve paralysis on the prognosis of the tuberculous pulmonary lesion. Some claim exceptionally satisfactory results (1, 2, 3). However, many of the less favorable results have not been reported. Little information is available correlating results with duration and type of disease.

The evaluation of any collapse procedure in pulmonary tuberculosis must in the strictest sense be based on control of the disease. It may be of secondary usefulness in temporarily halting the disease process for employment of other measures, or it may be palliative, adding to the patient's comfort.

To what degree and in what type of case is pneumoperitoneum successful?

In our opinion, the failure of agreement is not related to such factors as local prejudice, as has been stated editorially (4), but to the poor results in certain groups of cases. The results differ so markedly among the various types of disease that each should have its own valuation. Of the 382 cases reported by Trimble and Eaton (2), 37 per cent had exudative disease and relatively good results were obtained. In our series, most patients had fibrotic lesions and the results were poor. The extent of the lesions is important and should be stressed more often. In our series, 98 per cent of the patients had far advanced disease.

SUMMARY

This report presents the results obtained in the treatment of pulmonary tuberculosis. In the patients with predominantly exudative lesions, pneumoperitoneum appeared to be beneficial, although the number of cases, 16, is too small for accurate evaluation.

Pneumoperitoneum was found to be an unsatisfactory procedure in the patients who had the fibrotic type of disease of longer duration.

SUMARIO

Diez Años de Neumoperitoneo

Entre los enfermos aquí descritos, la formas exudativas de la tuberculosis pulmonar parecieron responder favorablemente al neumoperitoneo, aunque esta serie de 16 casos resulta insuficiente para justipreciación. El neumoperitoneo no es un procedimiento satisfactorio en la forma fibrosa de la dolencia ni en otras formas con lesiones muy antiguas.

RESUME

Dix années de pneumopéritoine

Dans les observations rapportées ici, les formes exudatives de la tuberculose pulmonaire paraissent réagir favorablement au pneumopéritoine, toutefois cette série de 16 cas est trop réduite pour permettre une appréciation exacte. Le pneumopéritoine ne constitue pas un procédé satisfaisant dans les formes fibreuses et dans les cas anciens.

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RIGHT-SIDED SPONTANEOUS PNEUMOTHORAX COMPLICATING THERAPEUTIC PNEUMOPERITONEUM

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Spontaneous pneumothorax complicating pneumoperitoneum therapy has been given little attention in the literature. The condition occurs *with or without loss of air* from the pneumoperitoneum. This report is concerned only with those instances of spontaneous pneumothorax complicating pneumoperitoneum in which there is simultaneous disappearance of the abdominal air. Although only 5 cases of this type have been reported (1–5), it seems likely that the condition is not as infrequent as has been thought. The authors have encountered 3 such cases, and reports of 12 additional cases were obtained in reply to a questionnaire sent to a group of chest physicians in California. In each of these cases the spontaneous pneumothorax occurred on the right side. The consistency of the right-sided location seemed sufficiently unusual to prompt investigation.

CASE REPORTS

Case 1: A 24-year-old student nurse was found to have active pulmonary tuberculosis in the left upper lobe in December 1945. Culture of a specimen of her sputum was positive for tubercle bacilli. Artificial pneumoperitoneum was initiated, and refills of 1,000 cc. of air were given every seven to ten days. The intraperitoneal pressures did not exceed +10 to +12 cm. of water. Treatment continued uneventfully until February 17, 1947. At that time the patient stated that, while she was having a bowel movement, she suddenly became short of breath and experienced substernal burning and palpitation. She noticed that her abdomen had become soft and flat. Roentgenograms demonstrated almost total collapse of the right lung with marked decrease in the size of the pneumoperitoneum (figure 1). The patient appeared only slightly dyspneic but was kept on strict bed rest. The right lung re-expanded slowly without further assistance.

In April 1947, therapeutic pneumoperitoneum was again initiated. Refills averaging 600 to 700 cc. of air were administered weekly, and intraperitoneal pressures did not exceed +10 to +12 cm. of water (figure 2). On September 2, 1947 the patient spent an hour stooping, kneeling, and bending in the garden while caring for her plants. Shortly afterward she noted an unpleasant sensation in her chest on taking a deep breath. A roentgenogram once more showed a large pneumothorax on the right. The pneumoperitoneum had disappeared (figure 3). Air was aspirated from the right pleural space, and the lung soon re-expanded. No further attempts were made to re-establish the pneumoperitoneum.

Case 2: A 58-year-old white male was found to have moderately advanced active pulmonary tuberculosis in October 1946. He was put on bed rest, and a left phrenemphraxis was performed. Following this, therapeutic pneumoperitoneum was initiated uneventfully. Refills of 800 to 1,000 cc. of air were given every seven to ten days. Intraperitoneal pressures did not exceed +10 to +12 cm. of water (figure 4).

On February 27, 1948, following a refill, the patient spent several hours cleaning his yard, lifting and carrying trash. Following this he complained of wheezing and moderately severe dyspnea. Fluoroscopy and roentgenograms showed a large pneumothorax and appar-

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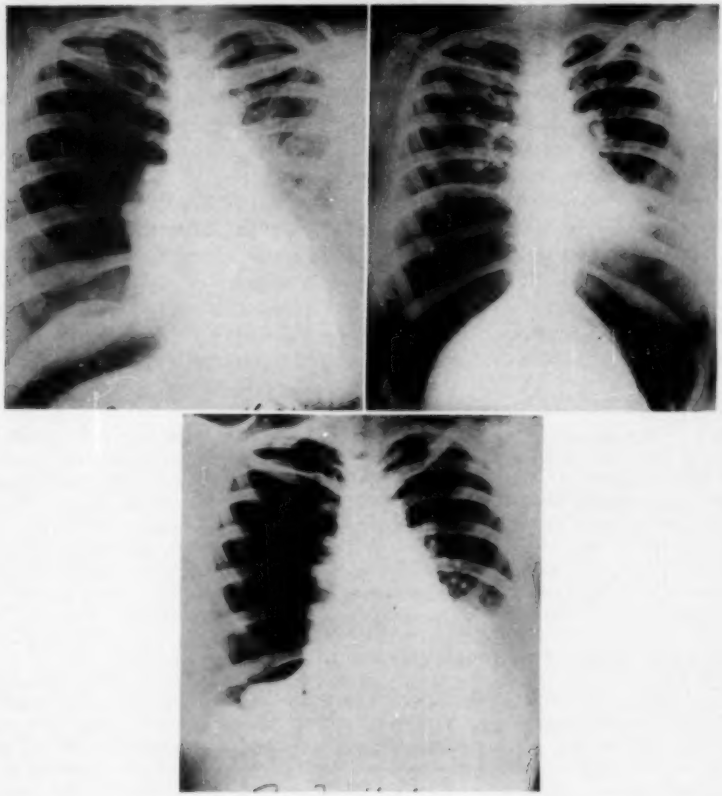
ent disappearance of the pneumoperitoneum (figure 5). Adhesion bands were seen extending from the right lung to the chest wall. A small amount of air was then injected into the peritoneal cavity in an attempt to demonstrate a communication between the right pleural



Case 1

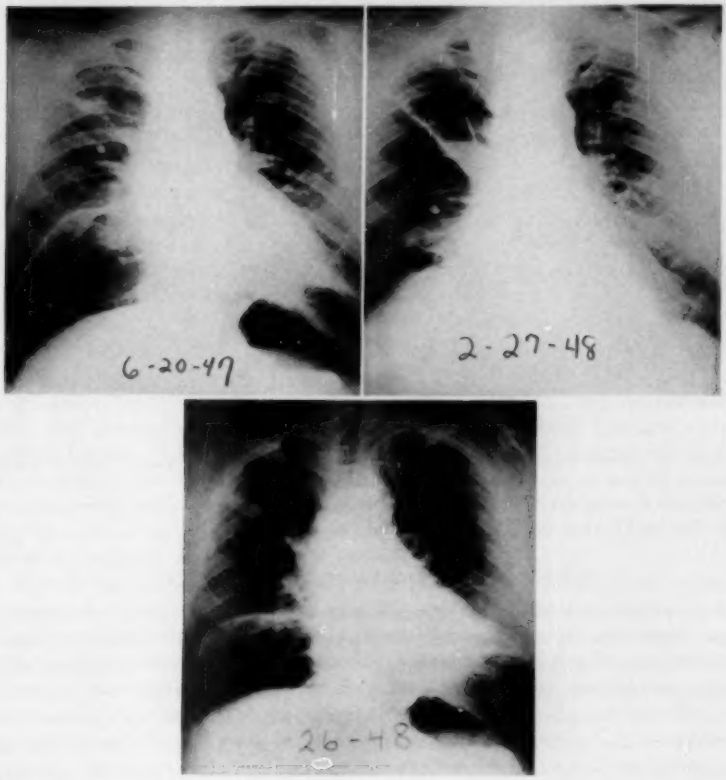
FIGS. 1-3. (Upper left) February 17, 1947. Tuberculous infiltrate in left second interspace. Right spontaneous pneumothorax present. Pneumoperitoneum greatly decreased in size.

(Upper right) June 30, 1947. Pneumoperitoneum re-established.

(Bottom) September 2, 1947. Second spontaneous pneumothorax on the right side. No pneumoperitoneum seen.

and peritoneal spaces. The air rapidly disappeared from the abdomen. Ten days later this was repeated. This time the pneumoperitoneum was successfully re-established (figure 6). The patient has continued asymptomatic for more than two years.

Case 3: A 20-year-old housewife was found to have bilateral active pulmonary tuberculosis in September 1946. Therapeutic pneumoperitoneum was started, and refills of 1,000 cc. of air were given weekly. Intraperitoneal pressures did not exceed +6 to +7 cm. of water (figure 7). On January 12, 1948, five days after a refill, the patient was seized with sudden



Case 3

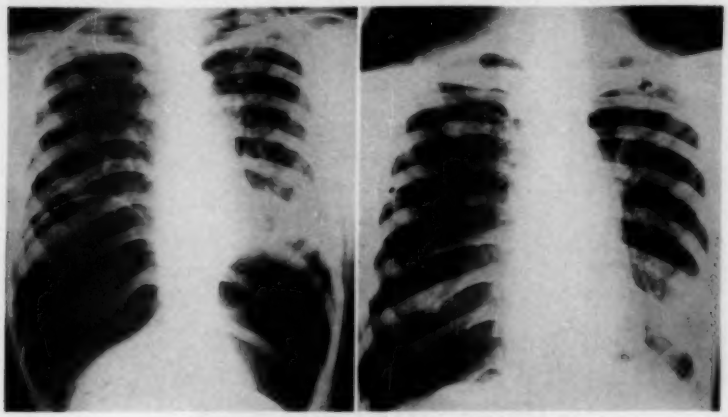
FIGS. 4–6. (Upper left) June 20, 1947. Bilateral tuberculous infiltrations in upper lobes. Moderately large pneumoperitoneum present.

(Upper right) February 27, 1948. Right spontaneous pneumothorax present. Dense adhesions noted. No pneumoperitoneum seen.

(Bottom) March 26, 1948. Pneumoperitoneum re-established.

oppressive pain in the right side of the chest and complained of extreme dyspnea, weakness, nervousness, and palpitation. Following this, she developed a nonproductive cough, wheezing, and fever. Fluoroscopy of the chest showed a spontaneous pneumothorax on the right, but no pneumoperitoneum was seen. Air was aspirated from the right pleural space

to alleviate the symptoms, and then a roentgenogram was made (figure 8). The pneumoperitoneum was abandoned, and the pneumothorax was maintained until progressive pleural symphysis obliterated the pleural space. In November 1948 pneumoperitoneum was started again. It has been continued since that time without incident.



Case 3

FIGS. 7–8. (Left) September 29, 1947. Bilateral pulmonary tuberculosis. Pleural density from old effusion on left. Large pneumoperitoneum present.

(Right) January 12, 1948. Right-sided pneumothorax present. Film taken following removal of air from chest. No pneumoperitoneum is noted.

RESULTS OF QUESTIONNAIRE AND CASE STUDIES

As previously mentioned, a questionnaire was sent to a group of California chest physicians in an effort to determine whether this complication was as uncommon as the paucity of reports in the medical literature would indicate. Sixty-one replies were received, and 12 cases of this syndrome were reported. In each case the spontaneous pneumothorax was on the right side and was associated with loss of the intra-abdominal air. In addition, there were reports of 8 instances of spontaneous pneumothorax *without* loss of air from the pneumoperitoneum, 7 of which were on the right side and one on the left.

Those physicians reporting cases of spontaneous pneumothorax associated with loss of abdominal air were sent a second questionnaire to determine what had been the final disposition of these patients. Pertinent data on their cases and on the 3 cases herein reported are recorded in table 1.

As may be noted in table 1, in 10 of the 15 patients, pneumoperitoneum was abandoned after the first episode of spontaneous pneumothorax. In one-half of these cases the spontaneous pneumothorax was continued as a therapeutic pneumothorax. Pneumonolysis was necessary in two instances.

Four of the 5 patients who had pneumoperitoneum re-established had a second episode of spontaneous pneumothorax. Two of these had a third such experience.

A second spontaneous pneumothorax was converted to a therapeutic pneumothorax with the aid of closed pneumonolysis in one instance. There was only one patient in whom pneumoperitoneum was re-established without a recurrence of spontaneous pneumothorax (two-year follow-up).

DISCUSSION

Two hypotheses have been advanced on the mechanism of loss of abdominal air in cases of spontaneous pneumothorax complicating therapeutic pneumoperitoneum. The first was advanced by Banyai and Jurgens (2) and later by Simmonds (3). It has been suggested that air from the pneumoperitoneum followed along the connective tissue sheath of the esophagus and great vessels through the diaphragmatic hiati into the mediastinum. Subsequently the increased tension of the accumulating air caused the pleural membrane to rupture, resulting in pneumothorax. In their cases, however, there was little supporting evidence of mediastinal emphysema in the signs or symptoms recorded. This is important, inasmuch as it has been emphasized (6) that spontaneous pneumothorax accompanying mediastinal emphysema is almost always located on the *left* side.

The second concept, predicated on the existence of a diaphragmatic defect, implies that the air from the pneumoperitoneum courses directly through it into the pleural space. These defects may be inflammatory (4, 7), congenital (1, 5, 8, 9), traumatic (10), or degenerative (11) in origin.

Inflammatory lesions of the diaphragm are usually secondary to a subdiaphragmatic abcess (10). These are more common on the right side. However, in none of the authors' cases was such a lesion noted.

Defects of the diaphragm may result from its imperfect development. These are due either to failure of formation of portions of its embryologic segments or to their improper fusion (10). Our knowledge of these diaphragmatic defects has largely resulted from fundamental studies of hernias of the diaphragm. In this connection, Harrington reported that the three commonest congenital diaphragmatic hernias are: those going through the pleuroperitoneal hiatus (Bochdalek's foramen), those resulting from lack of formation of a posterior portion of the diaphragm, and those passing anteriorly through the foramen of Morgagni (Larrey's space). The first two lesions are usually present at birth and commonly, but not invariably, result in large defects. They are most often found on the left side. When these defects are large, they frequently cause death of infants, due to interference with the function of the heart or lungs by the herniating organs. Furthermore, these large hernias have no pleural or peritoneal lining and have no sac.

Contrariwise, hernias occurring through the foramen of Morgagni are usually found in adults and are most often noted on the right side beneath the sternum and at the right costal margin (10). These hernias always have a hernial sac of peritoneum. This congenital defect would not allow re-establishment of a pneumoperitoneum without again resulting in a spontaneous pneumothorax.

As previously stated, congenital defects of the diaphragm are more common

on the left side. The right side is reinforced by the liver which bolsters defects above it, thus preventing omentum and small bowel from constantly knuckling into and enlarging these defects. This protective action obviously does not apply to very large lesions. These previously will have either resulted in death, have caused symptoms leading to discovery, or have been recognized by abnormal roentgenograms. Conversely, small defective areas remain occult when located in a portion of the diaphragm protected by the liver.

These defective areas lose their protection when pneumoperitoneum separates the liver and the diaphragm. For the first time they are subjected to the vicissitudes of great changes in intraperitoneal pressures. In contrast, these same forces have been exerted on the unprotected left diaphragm throughout the lifetime of the individual.

Because the liver gives some stability to the right diaphragm, hernias due to indirect trauma are also found more often on the left side. Harrington points out that these hernias are, in part, due to congenital inadequacies of the diaphragm. This is based on the observation that they usually occur at the line of fusion of its embryologic segments. Once the liver is separated from the diaphragm by pneumoperitoneum, the right diaphragm also becomes more susceptible to indirect trauma.

For completeness, it should be mentioned that atrophy of a portion of the diaphragm has been attributed to the use of pneumoperitoneum. It is, however, more commonly noted when phrenic crush is employed as an adjuvant to pneumoperitoneum therapy. The atrophic changes do not appear to be a likely cause for perforations of the diaphragm resulting in spontaneous pneumothorax. They do not explain the right-sided character of the lesion since phrenemphraxis is also employed on the left side; nor do these changes explain the infrequent occurrence of this complication in cases utilizing combined phrenic nerve surgery and pneumoperitoneum.

At thoracoscopy, Laird (11) noted vesicles or blebs on the upper surface of the diaphragm in 4 patients who were receiving pneumothorax and pneumoperitoneum concurrently. All 4 patients had paralyzed diaphragms preceding the use of pneumoperitoneum. Laird associated these blebs with peritoneal defects and with "the fibers of the diaphragm (being) stretched and thinned by paralysis."

Dr. J. Hallam Cope (12) performed a pneumonolysis on a patient who had had a spontaneous pneumothorax complicating his pneumoperitoneum therapy (table 1, case 4). In the course of the procedure, he inspected the diaphragmatic pleura through the thoracoscope. He noted a large bleb which varied in size with each respiration, enlarging whenever intra-abdominal pressure increased. This patient had had pneumoperitoneum for a relatively short time. No phrenic nerve operation had preceded its use.

These blebs may represent the herniation of peritoneum through a diaphragmatic defect, resulting in a pneumocele beneath the diaphragmatic pleura. This, then, is a potential precursor of a spontaneous pneumothorax, merely requiring a sufficient increase in intra-abdominal pressure to rupture the air sacs.

From the foregoing evidence, it seems clear that there are two principal factors to account both for the occurrence of this complication and for its uniformly right-sided appearance. The first is the presence of a diaphragmatic defect, usually congenital. The second is the dynamic action of the pneumoperitoneum pushing the liver and diaphragm apart, allowing unusual stress to bear on the right leaf of the diaphragm.

TABLE 1

Disposition of Patients with Spontaneous Pneumothorax and Loss of Pneumoperitoneum

CASES*	PNEUMOPERITONEUM		RECURRENCE OF SPONTANEOUS PNEUMOTHORAX	PNEUMOTHORAX MAINTAINED	REMARKS
	Abandoned	Re-established			
1	+				
2	+				
3		+	twice		Pneumoperitoneum abandoned after third spontaneous pneumothorax.
4	+			+	Pneumonolysis necessary
5	+				
6	+			+	
7	+			+	
8	+				
9		+	twice		Pneumoperitoneum abandoned after third spontaneous pneumothorax
10	+			+	Pneumonolysis necessary
11		+	once		Pneumothorax maintained; pneumoperitoneum abandoned after second spontaneous pneumothorax
12	+				
13		+	once		Pneumoperitoneum abandoned following second spontaneous pneumothorax
14		+			
15	+			+	Pneumoperitoneum re-established after loss of pneumothorax (pleural symphysis)

* The physicians reporting cases were as follows: Reginald H. Smart; Edward Dunner; James Duncan; Harold Trimble, Lloyd Eaton, *et al.*; Gordon A. Diddy; George S. Stephens; Sidney J. Shipman and Robert W. Clarke; Thomas B. Wiper; Buford H. Wardrip and Gerald Scarborough; James O. Greenwell. Cases 13, 14, and 15 are the authors' case reports.

The size of the defect in the *right leaf* of the diaphragm has a definite bearing on the temporal relationship of this complication to the initiation of the pneumoperitoneum. Patients having large diaphragmatic lesions would not be given pneumoperitoneum. Moderate-sized lesions would produce early appearance of a spontaneous pneumothorax. Smaller defects would become evident clinically when excessive intraperitoneal pressures were present (far and above those normally due to the pneumoperitoneum). Such pressures might result from straining at stool, persistent strenuous coughing, or the lifting of heavy objects. Potential defects, e.g., areas which have been poorly fused, may require the additional factor of indirect trauma to make them manifest.

Study of the questionnaire might suggest that spontaneous pneumothorax with loss of abdominal air was much more common than spontaneous pneumothorax without the loss of abdominal air among patients taking pneumoperitoneum. We are unable to state whether this observation is valid in view of the method of sampling employed. It is obvious, however, that once air has made its way from the peritoneal cavity to the pleural cavity, it will do so again in the majority of cases if the pneumoperitoneum is re-established. It is worth noting that spontaneous pneumothorax has been continued as a therapeutic pneumothorax in many instances. This is possible because the liver will once more abut the diaphragm when pneumoperitoneum is abandoned. Then, too, the air in the pneumothorax space is not under the same conditions of pressure as it is with pneumoperitoneum.

SUMMARY

Spontaneous pneumothorax complicating pneumoperitoneum therapy is not as infrequent a condition as the few published reports would indicate. In those cases in which the spontaneous pneumothorax has been associated with loss of intra-abdominal air, the pneumothorax always occurred on the right side. Fifteen previously unreported cases are added to the five already in the literature. The right-sided location of the spontaneous pneumothorax seems to be associated with the displacement of the liver incident to the use of pneumoperitoneum. Marked increase in intra-abdominal pressure or indirect trauma may be precipitating factors. Congenital defects of the diaphragm are believed to be the anatomic basis for this syndrome.

SUMARIO

Neumotórax Espontáneo del Lado Derecho como Complicación del Neumoperitoneo

El neumotórax espontáneo como complicación del neumoperitoneo terapéutico no es tan raro como indica la literatura. Si se asocia con la pérdida de aire abdominal, siempre se presenta en el lado derecho. A los 5 casos que ya figuran en la literatura, agréganse ahora 15 no comunicados todavía. La localización derecha del neumotórax espontáneo parece enlazarse con el desplazamiento del hígado, incidente al empleo del neumoperitoneo. El aumento pronunciado de la presión intraabdominal o el traumatismo indirecto pueden constituir factores precipitantes. Los vicios congénitos del diafragma pasan por formar la base anatómica del síndrome.

RESUME

Pneumothorax spontané droit compliquant un pneumopéritoine

L'apparition d'un pneumothorax spontané comme complication du pneumopéritoine thérapeutique n'est pas aussi rare que l'indique la littérature médicale. Quand le pneumothorax spontané s'accompagne d'une perte de l'air abdominal, le pneumothorax se produit toujours du côté droit. Quinze cas non encore publiés

s'ajoutent aux cinq déjà publiés. La localisation à droite du pneumothorax spontané paraît associé au déplacement du foie survenant à l'occasion du pneumopéritoine. Un accroissement marqué de la tension intra-abdominale ou un traumatisme indirect peuvent constituer des facteurs précipitants. La base anatomique de ce syndrome paraît être une malformation congénitale du diaphragme.

Acknowledgments

The authors are indebted to the many California chest physicians who were kind enough to respond to our questionnaires. Appreciation is expressed to Dr. Martin L. Stephenson for his kind advice and friendly criticism.

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THE VALUE OF SIMPLE VENTILATORY FUNCTION TESTS IN EVALUATING PATIENTS FOR THORACOPLASTY[1]

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INTRODUCTION

The need for some simple test for evaluating the pulmonary function of patients on whom thoracic surgery is anticipated has long been recognized. Cournand and Richards (1) have pointed out that pulmonary function has two principal divisions, ventilatory and respiratory. Ventilatory deficiency is mechanical in origin, the major symptom being dyspnea. Respiratory deficiency is physicochemical in origin, the major symptom being cyanosis. Warring (2) has demonstrated the practicability of simple procedures for evaluating the ventilatory function of patients with pulmonary tuberculosis prior to surgery. These procedures consist of: (*1*) a fluoroscopic examination of the patient, (*2*) an estimation of the *walking ventilation* of the patient expressed in liters per minute, and (*3*) an estimation of the *maximum breathing capacity* expressed in liters per minute. The ratio of $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ is next calculated. Values for this ratio of 0.35 or over signify poor ventilatory function and usually contraindicate surgery. A ratio of 0.50 indicates that the patient is a respiratory cripple. The apparatus required for these ventilatory function tests is relatively inexpensive, consisting of a 100-liter Douglas bag, a high velocity one-way valve, connecting hose and couplings, a gas meter, a stop watch, and face mask.

For the past three years these tests have been utilized by the authors in the routine evaluation of patients for thoracoplasty. In some patients bronchospirometry has also been performed. The purpose of the present report is to record the experience gained in the use of these tests and to confirm the results previously reported by Warring.

The Maximum Breathing Capacity (MBC)

Maximum breathing capacity: Maximum breathing capacity is obtained by having the patient breathe as forcibly and as rapidly as possible for thirty seconds through a one-way valve into a Douglas bag. The expired gas in the bag is measured by running it through the gas meter and the value thus obtained is multiplied by 2 and the result expressed as liters per minute. Two determinations are made and the higher figure taken as the patient's *maximum breathing capacity*. According to Warring, the normal *maximum breathing capacity* for a healthy male is approximately 150 liters per minute; and for a healthy female, approximately 100 liters per minute. Every effort must be made to encourage the patient to breathe as forcibly as possible into the bag during the test.

The Walking Ventilation (WV)

Walking ventilation: Walking ventilation is obtained by having the patient walk a distance of 180 feet in one minute while breathing easily through a one-way valve into

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the Douglas bag. The bag may be placed on a mobile table or it may be carried by the operator who times the patient with the stop watch. It is advisable to have the patient perform the test once with the valve open to become familiar with the pace and with breathing through the face mask. The test is then performed a second time with the patient breathing into the Douglas bag through the valve. Following the test the patient is questioned as to the presence or absence of dyspnea, and the gas in the bag is measured by running it through the gas meter. If the gas meter is calibrated in cubic feet, conversion to liters is obtained by multiplying by 28.3. In the present series, *walking ventilation* in various patients ranged from 9 to 25 liters per minute. In the majority of patients, Warring (3) found that the *walking ventilation* remained constant before and after the spread of disease, before and after clearing of disease, and before and after collapse therapy.

RESULTS

Ventilatory function tests were performed on 129 patients for whom thoracoplasty had been recommended or was being considered. A ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of 0.35 or over was considered to indicate a doubtful risk for surgery; a ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of 0.30 or over was considered borderline. Sixteen patients were rejected for surgery on the basis of poor ventilatory function. The other 113 patients had thoracoplasties.

Ten patients were operated upon who fell into the borderline and doubtful classes. Complications were encountered following thoracoplasty in one of the 7 borderline cases having ratios $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of greater than 0.30 but less than 0.35. No complications were encountered in any of the patients having ratios of less than 0.30. Two of 3 patients having ratios of 0.35 or over developed postoperative respiratory or cardiorespiratory complications. Summaries of the preoperative and postoperative findings of the 3 patients who developed such complications follow.

Case Reports

Case 1. This patient was a 22-year-old female who had bilateral pulmonary tuberculosis, classified on admission as far advanced in the right lung and moderately advanced in the left. She had a *walking ventilation* of 18 liters per minute, a *maximum breathing capacity* of 54 liters per minute, and a ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of 0.33. Following streptomycin therapy, the left side cleared and a right thoracoplasty was advised. Although studies showed the ventilatory function to be in the borderline class, it was decided to proceed with surgery in the hope that she would compensate adequately because of her youth. This proved not to be the case. Following each two-rib stage of an eight-rib thoracoplasty, she ran a stormy course, was cyanotic, and complained of dyspnea. Three months after her last stage she became markedly dyspneic, cyanotic, developed edema and other signs of cardiac decompensation, and died suddenly of cardiac failure.

Case 2. A second patient on whom a thoracoplasty was performed, in spite of a high ratio, was a 55-year-old male whose disease was classified as moderately advanced in the right

lung and minimal in the left. He had a *walking ventilation* of 19.7 liters per minute, a *maximum breathing capacity* of 52.5 liters, and a ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of 0.37. A seven-rib right thoracoplasty was completed, but one week after the last stage he developed signs of cardiac decompensation. He was digitalized and has been maintained on digitalis for nine months since his surgery. His present condition is fair although he still has considerable dyspnea.

Case 3. The third patient, who had a thoracoplasty in spite of a high ratio and who developed complications, was a 44-year-old male whose *walking ventilation* was 24.5 liters per minute. There was slight dyspnea on walking. The *maximum breathing capacity* was 66.0 liters per minute and the ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ was 0.37. Following the removal of six ribs in three stages, the patient became very dyspneic and cyanotic. Oxygen was administered for eighteen days postoperatively. The thoracoplasty was not continued. He was discharged one year later with his pulmonary disease inactive but with marked dyspnea on walking. Ventilatory function studies prior to discharge showed *walking ventilation* to be 27.1 liters per minute; *maximum breathing capacity* was 42.4 liters per minute; and the ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ was 0.66, indicating a respiratory cripple.

Warring reported no change in *walking ventilation* and a decrease in *maximum breathing capacity* of 3 to 30 liters in patients following thoracoplasty. In determinations on 31 patients in this study following thoracoplasty, an average rise of 1.2 liters per minute in *walking ventilation* was found following surgery. The magnitude of this rise, however, is within the limits of error for the test. There was an average decrease in *maximum breathing capacity* of 26.5 liters per minute and an average rise in the ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ of 0.13. One case showed improvement in *maximum breathing capacity* of 2.0 liters per minute following a six-rib thoracoplasty; all of the other cases showed decreases in *maximum breathing capacity* to 45 liters per minute.

DISCUSSION

It has been our experience that these simple tests which show the ventilatory ability of the patient, together with a careful fluoroscopic examination, are of great help in the evaluation of patients who are candidates for thoracoplasty.

It would seem practical to reject for surgery those patients in whom the ratio $\frac{\text{walking ventilation}}{\text{maximum breathing capacity}}$ is 0.35 or greater. As Warring has pointed out, severe dyspnea results if the level of the *maximum breathing capacity* falls below twice the value of the *walking ventilation*. The results obtained in this study would indicate that this is correct.

It must be emphasized that determination of *maximum breathing capacity* is far more accurate in estimating a patient's ability to ventilate than is the determination of vital capacity. In a patient with considerable emphysema, the inspiratory effort may be normal but expiration considerably prolonged. Such a patient, even with dyspnea, may have no notable diminution in vital capacity.

When *maximum breathing capacity* is performed, the sum of a series of respiratory efforts is determined and a much better estimate of a patient's ability to ventilate is obtained.

It is not meant to imply that other tests, such as determination of blood oxygen saturation before and after exercise and determination of residual air, are not valuable. On the contrary, these tests may occasionally indicate poor function in a patient in whom ventilation has been found to be normal. This is true in cases where there is interference with oxygen absorption. The equipment and staff required, however, for determination of blood oxygen and residual air places these tests out of reach of a majority of institutions. Warring's method can be used in any institution, and bronchospirometry can be performed in most. While Warring's method actually measures only ventilatory function, when ventilatory function is poor all aspects of pulmonary function are poor.

We are not prepared to say that the determination of the ratios between *walking ventilation* and *maximum breathing capacity* is an indispensable procedure and that it is more accurate than an accurate clinical evaluation of a patient whose clinical course is well known to the examining physician. We have been placing increasing reliance on Warring's method, however, particularly in our effort to confirm clinical impressions. In a patient who is refused surgery because of a low respiratory reserve, it is reassuring to all concerned to have clinical impressions backed by actual figures.

The surgeon who sees a large number of cases in consultation finds it practically impossible to evaluate personally all of the marginal risk patients. His decisions are likely to be more accurate if figures on pulmonary function are available.

SUMMARY

This report describes the methods of performing simple tests of ventilatory function (*maximum breathing capacity* and *walking ventilation*), and indicates the usefulness of these tests.

Utilizing the methods of testing suggested by Warring, 129 patients have been evaluated with ventilatory function tests prior to thoracoplasty. On the basis of the tests, 16 were rejected for surgery. Three patients known to have borderline or poor ventilatory function were subjected to operation, and all of the three developed respiratory or cardiorespiratory complications following thoracoplasty. Studies performed on 31 patients postoperatively served to confirm Warring's previous observations in the use of these tests following surgery.

SUMARIO

El Valor de Sencillas Pruebas de la Función Ventiladora en la Selección de Enfermos para la Toracoplastia

Descríbese el valor de ciertas sencillas pruebas de la función ventiladora (capacidad respiratoria máxima y ventilación ambulante). Ciento veintinueve enfermos fueron estudiados con pruebas de la función ventiladora antes de la toracoplastia, según propusiera Warring. A base de las pruebas, 16 de ellos

fueron rechazados para cirugía. Tres enfermos operados a pesar de saberse que la función ventiladora era dudosa o mala manifestaron complicaciones respiratorias o cardiorrespiratorias después de la toracoplastia. En 31 enfermos ejecutáronse estudios postoperatorios, confirmándose las previas observaciones de Warring con estas pruebas consecutivamente a la intervención cruenta.

RESUME

La valeur de simples tests de la fonction de ventilation pour l'évaluation des cas en vue de la thoracoplastie

La valeur des simples tests de la fonction de ventilation (capacité respiratoire maxima et ventilation au cours de la marche) est décrite.

Comme l'a suggéré Warring, l'évaluation préopératoire de 129 cas de thoracoplastie à l'aide des tests de la fonction de ventilation a été faite. D'après ces tests, 16 cas ont été écartés. Trois des malades ayant été opérés malgré une fonction de ventilation réduite ou constituant des cas limite, il s'est produit des complications respiratoires ou cardio-respiratoires à la suite de la thoracoplastie. Les études postopératoires effectuées sur 31 malades confirment les observations postopératoires précédemment faites par Warring à l'aide de ces tests.

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PERMANENCE OF SO-CALLED TEMPORARY PHRENIC NERVE PARALYSIS[1, 2]

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INTRODUCTION

Nearly forty years ago Stuertz (1) first produced paralysis of the hemidiaphragm as a therapeutic measure in pulmonary tuberculosis. His original operations consisted of division or partial resection of the main phrenic nerve trunk in an effort to produce permanent paralysis. A large proportion of patients subjected to this operation (phrenicotomy) continued to have normal diaphragmatic movement, or soon had return of diaphragmatic function. Felix (2) and Goetze (3), in 1922, demonstrated that in a large number of individuals there occur one or more accessory phrenic nerves which account for the failures of simple division. They devised operations to bring about complete and permanent paralysis by means of interrupting not only the main phrenic trunk but all accessory phrenic nerves as well. Felix advocated avulsion (phrenic exeresis) of the distal end of the nerve and its accessories by winding it around a hemostatic forcep. Friedrich (4), in 1914, was the first to point out the advantages of producing temporary rather than permanent diaphragmatic paralysis. His procedure was merely to crush the nerve (phrenemphraxis; phreniclasia).

There has been much controversy over the choice between permanent and temporary hemidiaphragmatic paralysis in the treatment of tuberculosis. Alexander (5) expressed the opinion that temporary paralysis in almost all cases is to be preferred to permanent paralysis, and this dictum is now rather universally accepted. Although the employment of phrenic nerve interruption as a sole procedure has become definitely less popular than in previous years, its usefulness as an auxiliary measure in certain cases is well established. The results obtained in the recent rather widespread employment of pneumoperitoneum, particularly when used in conjunction with unilateral temporary diaphragmatic paralysis, have served to arouse fresh interest in the operation of phreniclasia. Moyer (6) has pointed out recently the extreme value of this combination, especially in the group of patients seriously ill with bilateral pulmonary tuberculosis in whom the only alternative therapy would be bed rest. In a series of 550 patients, pneumoperitoneum was combined with temporary phrenic nerve interruption on the side of greatest involvement, and excellent results were obtained.

In the course of routine care of patients on a busy thoracic surgical service, the impression was gained that many patients having phrenic paralysis presumed

[1] From the Veterans Administration Hospital, Oteen, North Carolina.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

to be temporary in reality have had permanent phrenic paralysis. Because of this impression, it was decided to survey all patients in the hospital in whom temporary phrenic paralysis of the diaphragm had been instituted at some time during the course of their treatment in an effort to determine in what proportion the effect had been permanent.

COMPOSITION OF CLINICAL SERIES AND METHODS OF STUDY

Of a total of approximately 1,000 patients undergoing treatment for pulmonary tuberculosis, 288 previously had been subjected one or more times to the operation of phreniclasia at some period (335 operations). This group does not include those patients in whom the true result of the operation cannot be determined due to subsequent events (i. e., pneumonectomy, extensive thoracoplasty, chronic empyema). In the 288 patients, multiple operations were performed in 38 instances (secondary crush in 30 and secondary and tertiary crush in 8).

TABLE 1

Phreniclasia: Present Status of Diaphragm in 157 Patients Subjected to Temporary Phrenic Nerve Interruption More than One Year Previously

STATUS OF DIAPHRAGMATIC FUNCTION	NUMBER OF CASES	PERCENTAGE OF TOTAL GROUP
Complete paralysis	29	18.5
75 per cent paralysis	13	8.3
50 per cent paralysis	15	9.5
25 per cent paralysis	27	17.2
Total showing partial or complete paralysis	84	53.5
Total showing no paralysis	73	46.5

In 162 patients, operation was on the right side; and in 126 cases, it was on the left side. Fluoroscopic examinations of all patients were made in the erect position, and the diaphragmatic response to a short inspiratory sniff was noted. The degree of paralysis of the involved hemidiaphragm was estimated as complete (100 per cent), 75 per cent, 50 per cent, 25 per cent, and no paralysis. Of the entire group, 131 patients had been operated upon within a year subsequent to the time of the study. These cases were discarded as being too recent for true evaluation, and the remaining 157 were subjected to analysis.

OBSERVATIONS

The results of this evaluation are shown in table 1. As may be seen, among those patients in whom presumably temporary phrenic nerve interruption had been performed more than a year previously, more than half had partial or complete paralysis of the hemidiaphragm at the time of examination. It may be mentioned here also that, of the 131 recent phrenic operations performed during the past year, 69 showed 100 per cent paralysis, 23 had 75 per cent paralysis, 8 had 50 per cent paralysis, 9 had 25 per cent paralysis, and 22 had completely regained function (no residual paralysis).

DISCUSSION

Trimble and associates (7) in a recent communication stressed the fact that phrenic operation is not an entirely innocuous procedure, and that permanent interference with the function of the diaphragm frequently occurs even after so-called temporary nerve blocks. Our investigation tends to substantiate this statement and our previous clinical impression. The operations in this series were performed by a number of surgeons employing more or less similar technique with only minor variations. It is not the purpose of this paper to discuss details of the operative procedure or of individual variations in technique. It may be said, however, that the technique employed consisted of exposing the phrenic nerve through a transverse cervical incision and crushing the nerve for a distance corresponding to the width of one to three hemostats. The brachial plexus was exposed and any accessory phrenics were divided. The degree of paralysis was verified by postoperative fluoroscopy.

The observations indicate that in any large series of patients subjected to temporary phrenic paralysis there will occur a certain number in whom varying degrees of permanent interference with diaphragmatic function results. As would be expected, the degree of permanent disability was correspondingly higher in those patients who underwent multiple phrenic nerve operations. In this series, 84 of 157 patients (53.5 per cent) showed some degree of residual paralysis more than a year later, varying from 25 to 100 per cent; and 29 of 157 (18.5 per cent) were still completely paralyzed. These figures are somewhat higher than those usually quoted.

In terms of pulmonary functional disability, figures such as these must be weighed carefully when phreniclasia is being considered as a therapeutic procedure.

SUMMARY

This report summarizes an analysis of 288 patients previously subjected to phreniclasia (335 operations) for temporary phrenic nerve paralysis, in an effort to determine the permanence of the resulting paralysis. The observations in 288 patients indicated the occurrence of some degree of permanent paralysis in approximately one-half of the cases and total loss of diaphragmatic function in almost one-fifth.

SUMARIO

En una serie considerable de operaciones practicadas para obtener parálisis temporal del frénico, hay que estar preparado para observar alguna parálisis permanente aproximadamente en la mitad de los casos y pérdida total de la función diafragmática casi en la quinta parte de los mismos.

RESUME

Dans une longue série d'interventions pour des cas de paralysie temporaire du phrénique, il y a lieu de s'attendre à un certain degré de paralysie permanente dans la moitié des cas environ et la perte totale de la fonction diaphragmatique dans un-cinquième des cas.

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ANTIGENIC ACTIVITY OF FRESH, FROZEN, AND DRY BCG VACCINE[1]

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INTRODUCTION

Our previous study (1) with freeze-dried BCG vaccine clearly demonstrated the ability of such a preparation to retain its antigenicity for at least one year under adverse physical conditions. It also proved that, in spite of its greatly reduced living population, the surviving organisms retain their original morphologic, cultural, and biologic characteristics. It seemed reasonable, therefore, to suppose that the observed lower antigenicity of dry vaccine, as demonstrated by smaller skin lesions with graded doses of vaccine, the diminished degree of tuberculin sensitivity, and the lower resistance against a challenge infection with virulent tubercle bacilli, is mainly due to the proportionally lower content of living elements in the dry vaccine as compared with the number of living elements in the freshly prepared liquid vaccine. The study suggested that, with proper adjustment of the dosage of living elements in the resuspended dry vaccine, one should be able to induce antigenic responses identical with those following the use of fresh vaccine.

The present study details the results obtained in guinea pigs immunized with one-year-old lots of frozen and of dry BCG vaccine and subsequently challenged with virulent tubercle bacilli. The multiple-puncture method of vaccination was employed, using 40 punctures in one series and 160 in another. Control series included animals immunized with freshly prepared vaccine as well as nonimmunized animals.

Experimental Methods and Data

Preparation of frozen and of dry BCG vaccines: Pure 10-day-old BCG Sauton II cultures (A-38, June 7, 1948) were pressed into a semidry mass in our harvesting apparatus (2) and finely divided in the ball-mill with inoxidable steel balls of diameters of 4 mm. and 3 mm. for five minutes at 60 r.p.m. Sufficient 50 per cent glucose solution was added to make a BCG suspension of 200 mg. per ml., and rotation was continued for ten minutes. At this time, microscopic examination of the suspension revealed finely dispersed, individual acid-fast rods interspersed among minute clusters containing varying numbers of BCG, most of them with less than one hundred and occasionally with as many as several hundred organisms. Attempts to assess the actual numbers of viable BCG per mg. of finely divided vaccine suspensions would, therefore, seem futile by cultural methods. For comparative purposes, however, such standardized procedure will yield parallel counts of colonies. Viability tests with 10^{-6}, 10^{-6}, and 10^{-7} dilutions of the freshly prepared BCG A-38, suspended in 50 per cent glucose and seeded out in 0.2 ml. amounts in 50 ml. tubes

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of Löwenstein's medium, gave rise to an average of 47 million colonies per mg. of semidry culture.

The 200 mg. per ml. BCG glucose vaccine was immediately aspirated in 30 ml. amounts into sterile flasks and quickly frozen in a dry-ice alcohol bath under constant rotation. The material adhered to the sides of the flask in a thin crystalline layer. For comparative purposes, five such flasks were promptly placed at −20°C. for further study of the effect of this procedure on BCG's viability and antigenic activity. The remaining flasks were connected with a lyophile apparatus and the vacuum pump was started. Three minutes later, the pressure in the flasks registered less than 0.1 mm. of mercury, and it was continued at that level for the 36-hour lyophilization run when the glucose BCG material appeared sufficiently dry. The flasks were sealed with rubber stoppers under vacuum and twenty-four hours later their condition was found satisfactory. At that time, the moisture in one flask was 2.11 per cent when dried at 100°C. under vacuum over P_2O_5 to constant weight. The frozen vaccine flasks were kept constantly at approximately −20°C. and the dry glucose vaccine flasks at approximately 2° to 4°C.

TABLE 1

Survival of Living Elements in Frozen and in Dry BCG Vaccines

Number of Colonies per Mg. Semidry Weight of Resuspended Vaccines and Percentage Survival at Various Intervals over a Period of One Year*

TYPE OF VACCINE	AGE OF VACCINE					
	Fresh	2 days	3 months	6 months	9 months	12 months
Frozen	47,000,000	19,000,000 (40.43)	2,600,000 (5.74)	2,100,000 (4.47)	2,050,000 (4.36)	1,800,000 (3.81)
Dry	47,000,000	8,000,000 (17.02)	7,400,000 (15.74)	7,200,000 (15.32)	7,000,000 (14.89)	6,500,000 (13.83)

* The figures in parentheses represent the percentage survival of living elements.

VIABILITY OF BCG IN FROZEN LIQUID AND IN DRY PREPARATIONS

Viability tests were repeated at intervals of two days and three, six, nine, and twelve months after freezing and drying. Both the frozen and the dried materials were readily resuspended in phosphate buffer solution (pH 7.3). The usual serial dilutions were seeded out in 0.2 ml. amounts on 50 ml. tubes of Löwenstein's medium. The tubes were left in horizontal position at 38°C. for approximately four hours until the liquid had evaporated. At this time the tubes were sealed with rubber stoppers and inverted. Readings of colonies were made one and two months later. The results are recorded in table 1. It is apparent from the data in table 2 that the process of lyophilization destroys more living BCG than does the method of simple rapid freezing, but that the dry vaccine remains relatively stable in living elements when stored at 2° to 4°C. up to one year; during the same period the frozen vaccine continues to lose its living elements.

The freshly prepared BCG vaccine (A-64, June 5, 1949) yielded 53 million living aggregates (colonies) per mg. of semidry weight, or approximately the same living population as in the original lots of frozen and dry vaccine.

TABLE 2

Tuberculous Hyperplasia in Animals Immunized with Fresh, Frozen, and Dry Lots of BCG

Weights in Percentage of Body Weight of Abscess, Lymph Nodes, Spleen, Liver, and Lungs Statistically Analyzed against Controls

ORGANS	CONTROLS	FRESH VACCINE						DRY VACCINE						FROZEN VACCINE					
		40 punctures			160 punctures			40 punctures			160 punctures			40 punctures			160 punctures		
	Average	Average	t	P	Average	t	P	Average	t	P	Average	t	P	Average	t	P	Average	t	P
Body weight in grams	946	789	3.896	<0.001	816	4.234	<0.001	777	2.863	<0.01	812	5.963	<0.001	791	3.496	<0.01	793	4.717	<0.001
Per cent of body weight																			
Subcutaneous abscess	.396	.068	3.054	<0.01	.009	3.812	<0.001	.188	2.385	0.03	.013	3.737	<0.001	.280	1.643	0.15	.053	11.034	<0.001
Superficial inguinal glands	.360	.151	3.989	<0.001	.051	6.095	<0.001	.141	3.631	<0.001	.129	3.373	<0.01	.221	2.405	0.03	.168	3.790	<0.001
Deep inguinal glands	.060	.064	3.212	<0.01	.013	4.161	<0.001	.099	2.306	0.03	.033	2.948	<0.01	.065	1.245	0.21	.042	2.634	0.02
Iliac glands	.333	.142	4.240	<0.001	.037	8.348	<0.001	.124	4.505	<0.001	.088	6.872	<0.001	.228	2.252	0.04	.139	4.295	<0.001
Periportal glands	.196	.061	5.112	<0.001	.009	10.167	<0.001	.040	6.784	<0.001	.001	7.023	<0.001	.137	2.867	0.02	.041	7.261	<0.001
Tracheobronchial glands	.478	.189	6.652	<0.001	.064	11.703	<0.001	.171	6.136	<0.001	.053	12.965	<0.001	.384	1.113	0.28	.184	5.873	<0.001
Total glands	2.126	.705	6.066	<0.001	.205	27.873	<0.001	.652	5.645	<0.001	.433	5.430	<0.001	1.272	4.081	<0.001	.804	5.406	<0.001
Spleen	.631	.218	3.915	<0.01	.114	3.206	<0.01	.196	2.867	0.02	.107	3.339	<0.01	.238	2.411	0.03	.159	2.048	<0.01
Liver	5.740	4.945	1.843	0.11	4.704	2.981	<0.01	5.273	1.229	0.25	4.954	2.181	0.03	5.260	1.255	0.19	5.655	0.221	0.82
Lungs	1.870	1.388	1.890	0.15	.948	4.182	<0.001	1.273	1.406	0.18	1.079	3.394	<0.01	1.252	1.778	0.09	1.071	3.021	<0.01
Per cent of significant deviations:			81.82		100				54.55		90.90				18.18		81.82		
			90.91%						72.73%						50.00%				

N. B. In Fisher's 1936 tables for distribution of t (quotient expressing the deviations as a multiple of its probable error), we observe that a $P \leqslant 0.01$ (probability that the observed mean deviation bearing this or smaller values cannot have occurred by chance alone) requires that $t \geqslant 2.819$ when each group contains 12 animals or samples. Every difference having *absolute significance* is italicized.

ANTIGENICITY OF FRESH, FROZEN, AND DRY BCG VACCINE

Transcutaneous skin lesions: Seventy-two normal and tuberculin-negative white guinea pigs, weighing 382 sigma 25.27 gm., were epilated with electric clippers on the right and left sides of the body. The skin was washed with alcohol and ether. In groups of 12 animals, the following procedure was followed in vaccinating the animals with the 20 mg. per ml. resuspended one-year-old lots of frozen and dry BCG A-38 vaccines: Holding the skin taut, sufficient vaccine was spread evenly across the epilated area; and 40 punctures, approximately 3 mm. in depth, were made through the skin of 12 animals with our automatic, spring-actuated, multiple-puncture vaccination apparatus (3); 160 punctures were made in 12 others. The skin was stretched slightly to admit as much vaccine as possible into the punctures. Twelve other guinea pigs were given 40 punctures through the skin moistened with freshly prepared BCG A-64 containing 20 mg. per ml. of BCG, and a similar group was given 160 punctures. These groups will henceforth be called Group 1a (fresh vaccine—40 punctures); Group 1b (fresh vaccine—160 punctures); Group 2a (dry vaccine—40 punctures); Group 2b (dry vaccine—160 punctures); Group 3a (frozen vaccine—40 punctures); and Group 3b (frozen vaccine—160 punctures).

During the following twelve weeks, measurements were made weekly of the maximum diameters of the skin vaccination nodules in these six different groups. Only one week after vaccination, a marked difference was obtained in the inflammatory response to the three types of vaccine. Groups 1a and 1b (fresh vaccine) presented red papules, 2 mm. wide, which were raised markedly in the centers without any suppuration. Groups 2a and 2b (dry vaccine) presented papules, 1.0 to 1.5 mm. wide and less intensely red, while Groups 3a and 3b (frozen vaccine) showed barely visible tawny-colored flat points, 1 mm. wide. Three weeks after vaccination, those animals given the fresh vaccine exhibited papules 3.0 to 3.5 mm. wide, markedly elevated, with occasional yellow-ocher centers, but without any frank suppuration. Those given the dry vaccine had intensely red papules, 2.5 to 3.0 mm. wide, without signs of necrosis. At the same time, the group given frozen vaccine showed slightly less intensely inflamed papular skin reactions, 2.0 to 2.5 mm. in width. Six weeks after vaccination, Groups 1a and 1b (fresh vaccine) presented almost unchanged intense local reactions, with suppuration from many papules, desquamation, and thickening of the underlying skin. Adjacent inguinal and axillary lymph nodes were definitely palpable. At this time Groups 2a and 2b (dry vaccine) presented less intense local reaction, with desquamation nearly completed, suppuration less frequent, and skin almost as thickened as in the animals given fresh vaccine, although the adjacent lymph nodes were less enlarged. In the group given frozen vaccine, local reactions had almost completely healed and the skin was only slightly thickened. The adjacent lymph nodes were definitely enlarged. Twelve weeks after vaccination, Groups 1a and 1b (fresh vaccine) still presented visibly elevated and slightly injected papules, while the adjacent lymph nodes were enlarged and of hard consistence. At this time Groups 2a and 2b (dry vaccine) presented barely visible whitish nodules; the skin was of normal thickness and the adjacent lymph nodes were

slightly enlarged but of soft consistence. The group given frozen vaccine showed no residual local lesions, the skin was supple, and the local glands slightly enlarged and soft.

The general impression gained from the transcutaneous skin lesions produced by these different types of vaccines was that the dry BCG excites slightly smaller local reactions than the freshly prepared vaccine, but that the frozen BCG vaccine preparation produces the least and most transitory local skin reactions.

Tuberculin sensitivity: Differences in the antigenicity of the three vaccines are readily demonstrated by the rapidity and intensity with which tuberculin sensitivity is produced in the vaccinated animals. Figure 1 shows the degree of allergy[2] with 10 mg. OT injected intracutaneously four weeks after vaccination. All of the 24 animals given the fresh vaccine reacted positively, with 260 ± 194.10 mm.[3] induration in 48 hours in Group 1a, and with 705 ± 302.81 mm.[3] in Group 1b. At this time, only 7 of the 12 animals in Group 2a reacted positively with 67 ± 71.93 mm.[3] induration, and 10 of 12 animals in Group 2b reacted with 166 ± 69.18 mm.[3] In Group 3a, only 4 of 12 animals reacted with an insignificant reaction measuring 9 ± 5.92 mm.[3], and in Group 3b only 7 of 12 animals reacted with 49 ± 37.90 mm.[3]

Eight weeks after vaccination the tuberculin tests were repeated with 1.0 mg. OT. All animals in Groups 1a and b reacted positively, with 131 ± 61.97 mm.[3] induration in the former, and 430 ± 165.59 mm.[3] in the latter. The same held true in Groups 2a and b. In the former, there was 111 ± 55.08 mm.[3] induration and in the latter 226 ± 53.41 mm.[3] In Group 3a only 8 animals reacted with 93 ± 72.92 mm.[3] induration, and in Group 3b only 9 animals reacted with 161 ± 94.65 mm.[3] induration.

Twelve weeks after vaccination, the tuberculin tests were repeated with 0.1 mg. OT. Groups 1a and b now reacted intensely and uniformly with 344 ± 130.61 mm.[3] and 538 ± 193.30 mm.[3] induration, respectively. In Group 2a, 11 animals reacted with 225 ± 110.33 mm.[3] induration, and all animals in Group 2b showed 424 ± 154.06 mm. induration. In Group 3a, 10 animals reacted with 178 ± 108.94 mm.[3] induration, and all the animals in Group 3b reacted strongly with 303 ± 202.50 mm. induration.

Again it becomes apparent that the dry BCG vaccine lags decidedly behind the freshly prepared vaccine in its ability to sensitize the tissues to tuberculin, and that the frozen vaccine is even weaker in this respect than the dry vaccine. Careful scrutiny of figure 1, however, makes it apparent that by quadrupling the vaccination punctures with the dry and frozen vaccines, the resulting tuberculin sensitivity is almost comparable with that produced by only 40 punctures when the freshly prepared vaccine is used.

It should be mentioned here that the weight curves in these six groups of animals were approximately parallel 12 weeks after vaccination. The average

[2] Expressed in cubic millimeters of induration, calculated as half the thickness of skin folded between caliper prongs multiplied by the two surface diameters of the tuberculin-indurated area. The dispersion measure following the ± sign is the standard deviation of the mean as used by R. A. Fisher (5).

weight of the 72 animals had increased from 382 ± 25.27 gm. to 628 ± 44.74 gm.

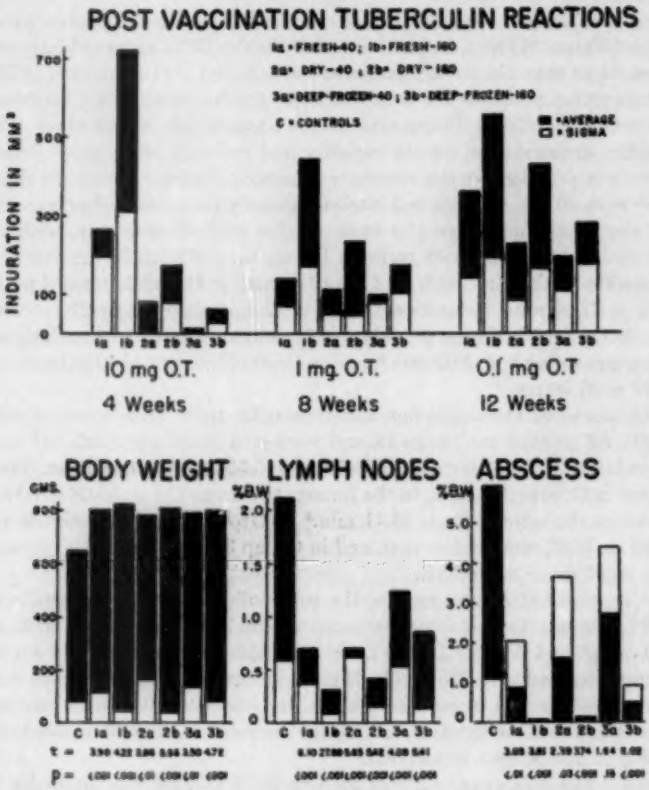

FIG. 1. Post-vaccination tuberculin reactions and body weights.

PROTECTIVE VALUE OF A FRESH, FROZEN, AND DRY BCG VACCINE AGAINST A CHALLENGE INFECTION

Three months after vaccination, the six groups of variously inoculated animals and 18 normal and nonimmunized control animals, weighing 615 ± 18.56 gm., were inoculated subcutaneously in the left hind leg with 0.0005 mg. semidry weight of a one-month-old Löwenstein egg-medium culture of a virulent strain of human tubercle bacilli (No. 5625). One-tenth mg. semidry weight of this culture, isolated from the sputum of a patient with pulmonary tuberculosis, had killed a guinea pig with generalized tuberculosis four weeks after subcutaneous inoculation in the left hind leg. The challenge infective dose yielded 1,084 colonies on Löwenstein's medium.

Two months after the virulent inoculation, tuberculin tests with 1.0 mg. OT in the 18 control animals produced 1,453 ± 69.84 mm.² induration after 48 hours. At this time the adjacent left superficial inguinal glands were definitely enlarged and of hard consistence. The same OT dose produced significantly less induration in all of the six groups of vaccinated animals, while the left superficial inguinal glands adjacent to the site of virulent inoculation were only vaguely palpable in Group 3a. Presumably the delayed production of tuberculin hypersensitiveness in the immunized groups following the virulent inoculation is referable to the mechanical barrier produced by BCG in reducing the multiplication and spread of the challenge infection.

Four months after the virulent inoculation, the weight curves gave definite indication of the state of tuberculous infection in the variously vaccinated and in the nonvaccinated control animals. The weights were as follows: Group 1a, 880 ± 76.16 gm.; Group 1b, 884 ± 69.92 gm.; Group 2a, 817 ± 75.20 gm.; Group 2b, 840 ± 84.65 gm.; Group 3a, 785 ± 89.29 gm.; Group 3b, 732 ± 74.20 gm., and the nonvaccinated control group, 715 ± 101.66 gm. The differences in body weights between the control animals and the Groups 1a and b (fresh vaccine) and Groups 2a and b (dry vaccine) were significant while those of Groups 3a and b (frozen vaccine) were insignificant.

Similar differences were obtained in the variously vaccinated groups, in the subcutaneous abscesses on the left hind legs of the control animals (site of virulent inoculation), and adjacent superficial and deep inguinal glands which were proportionally of greater size in the control animals and in Groups 3a and b (frozen vaccine) than in the rest of the vaccinated animals. The least enlargement was observed in the animals vaccinated with fresh vaccine.

Mortality rate of immunized and control animals: Among the 18 nonvaccinated control animals, 11 (61.1 per cent) died spontaneously with generalized tuberculosis between 121 and 174 days after the virulent inoculation. In Group 1a, 2 animals died (16.7 per cent) 122 and 147 days, respectively, after the virulent inoculation, while none died spontaneously in Group 1b. In Group 2a, 3 died (25 per cent) 133, 157, and 165 days, respectively, after the virulent inoculation, while no spontaneous deaths occurred in Group 2b. In Group 3a, 5 died (41.7 per cent) between 118 and 171 days after the virulent inoculation, while none died spontaneously in Group 3b. All of the surviving animals were killed for comparative study 174 and 178 days after the inoculation with virulent human tubercle bacilli.

Thus it would appear that, in comparison with the nonvaccinated control animals, Group 3a (frozen vaccine—40 punctures) showed the poorest survival rate and Group 2a (dry vaccine—40 punctures) fared slightly better. No apparent difference exists in the survival rate of the three groups of animals given 160 punctures through the skin moistened with 20 mg. per ml. semidry weight of either fresh or 12-month-old frozen or dry BCG vaccine.

Quantitative analysis of delayed tuberculous hyperplasia: The same autopsy procedure was adhered to as on previous occasions (4) and the completed data, converted into percentage of the animal's body weight, were tested for significance by Fisher's method (5) for comparison of two means (table 2 and figure

2). These data make it apparent that the best protection against a virulent challenge infection is afforded by the freshly prepared BCG vaccine and that the significant results obtained with 40 punctures (81.82 per cent) are strikingly enhanced by the use of 160 punctures (100 per cent). Next in order of protective value is the one-year-old dry BCG vaccine which, following the use of 40 punc-

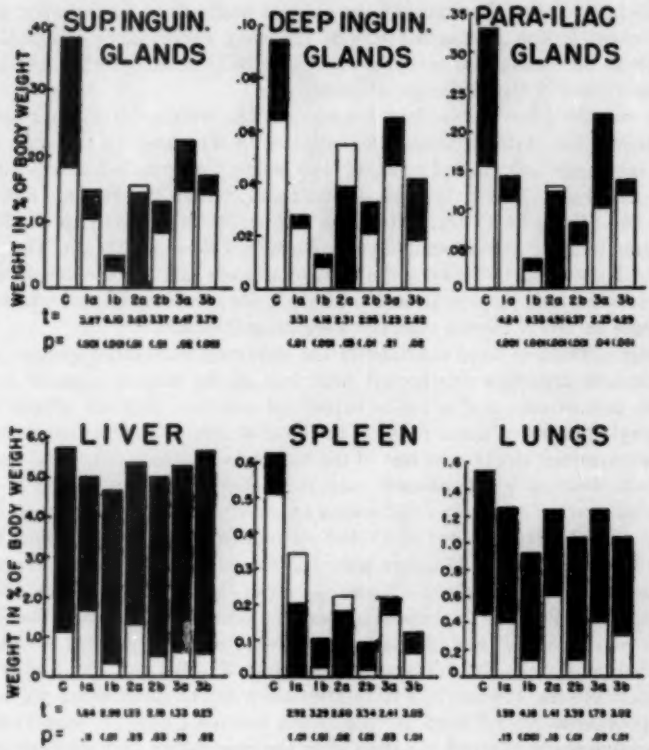

FIG. 2. Tuberculous hyperplasia in lymph glands and in the spleen, liver, and lungs.

tures, yields 54.55 per cent significant deviations in tuberculous hyperplasia from those observed in the nonvaccinated controls; by increasing the punctures to 160, the protective value of the dry vaccine scores 90.90 per cent significant deviations, which is more than is observed with fresh vaccine and 40 punctures. The poorest protective value is shown with one-year-old frozen BCG vaccine and 40 punctures (18.18 per cent significant deviations) but again, by increasing the punctures to 160, the immunizing result is exactly comparable (81.82 per cent significance) with that obtained with fresh vaccine and 40 punctures.

These investigations should stimulate further application in man of dry and frozen BCG vaccine, preferably with maximum doses or repeated inoculations, since both the dry and the frozen vaccines appear to possess qualitative antigenic activities identical with those of the freshly prepared vaccine, despite the fact that their population of living elements is markedly less than that in the freshly prepared vaccine. The advantage of the dry or frozen BCG vaccine is that its distribution for use in man can be properly controlled for purity, viable microorganisms, tuberculogenic activity, and absolute harmlessness many months after its preparation; whereas at present a brief 10-day period of optimum antigenic activity is prescribed for freshly prepared liquid BCG vaccine.

CONCLUSIONS

1. Glucose BCG vaccine sustains a greater loss of living organisms during the prolonged process of drying from the frozen state than during the quick process of simple freezing as an alternative means of preservation.

2. Dry glucose BCG vaccine retains its surviving organisms at a relatively stable numerical level for one year when kept at 2° to 4°C., while frozen glucose BCG vaccine gradually dissipates its living organisms to a lower numerical level than the dry vaccine when kept at −20°C. for one year.

3. The morphologic, cultural, and biologic characteristics of surviving one-year-old dry and frozen BCG remain unaltered from those possessed by fresh liquid vaccine.

4. The antigenicity of one-year-old dry glucose BCG vaccine is lower than that of fresh liquid vaccine. Moreover, the one-year-old frozen glucose vaccine is weaker antigenically than similarly aged dry glucose vaccine as demonstrated by skin lesions, tuberculin sensitivity, and resistance against a challenge infection with virulent tubercle bacilli.

5. By quadrupling the multiple skin punctures, the use of one-year-old vaccine, either the dry glucose BCG vaccine or the frozen glucose vaccine, produces a degree of resistance against a challenge infection with virulent tubercle bacilli comparable to that stimulated by one-fourth the number of skin multiple punctures and the use of fresh liquid vaccine.

6. With adequate adjustment of multiple skin punctures and dosage with reference to living organisms or repeated inoculations, the use of one-year-old and completely controlled dry or frozen BCG vaccine should prove just as effective as the use of fresh liquid vaccine. Moreover, the dry or frozen vaccine seems preferable to fresh liquid vaccine because the latter preparation is incompletely controlled, and maintains optimal antigenic activity for only ten days after its preparation.

CONCLUSIONES

Actividad Antigénica de las Vacunas BCG Fresca, Congelada y Seca

1. La vacuna BCG glucosada experimenta una pérdida mayor de microorganismos vivos durante el prolongado proceso de desecación-congelación que durante el rápido proceso de congelación profunda.

2. La vacuna BCG glucosada seca retiene los microorganismos sobrevivientes a un nivel numérico relativamente estable durante un año si se conserva a una temperatura de 2° a 4° C., mientras que la glucosada congelada disipa sus microbios vivientes a un nivel numérico más bajo que la seca, cuando se conserva a −20° C. por un año.

3. Las características morfológicas, culturales y biológicas de los BCG sobrevivientes de un año y congelados no discrepan de las poseídas por los de la vacuna líquida fresca.

4. La antigenicidad de la vacuna BCG glucosada seca de un año es menor que la de la vacuna líquida fresca y la vacuna glucosada congelada de un año es más débil antigénicamente que la glucosada seca de edad semejante, según demuestran las lesiones cutáneas, la sensibilidad a la tuberculina y la resistencia a una infección provocadora con bacilos tuberculosos virulentos.

5. Cuadruplicando las multipunciones cutáneas, el empleo de vacuna BCG glucosada seca y de vacuna glucosada congelada, ambas de un año, producen una resistencia a una infección provocadora con bacilos tuberculosos virulentos, comparable a la excitada con la cuarta parte del número de multipunciones cutáneas cuando se emplea vacuna líquida fresca.

6. Ajustando adecuadamente el número de multipunciones cutáneas y la dosis con referencia a bacilos vivos o a repetición de inoculaciones, el empleo de vacuna BCG glucosada de un año y absolutamente comprobada, ya seca o congelada, debe resultar igualmente eficaz que el, y preferible al, de la vacuna líquida fresca incompletamente comprobada que disfruta, después de su preparación, solamente un breve período de diez días de actividad antigénica óptima.

CONCLUSIONS

Activité antigénique des vaccins BCG frais, congelés et secs

1. Le vaccin BCG glucosé subit une perte de germes vivants plus grande au cours du procédé froid-dessication prolongé que pendant le procédé de congélation rapide.

2. Le vaccin BCG glucosé sec conserve son contenu en germes survivants à un niveau relativement stable pendant un an, quand il est conservé à une température de 2° à 4° C. Quant au BCG glucosé congelé, la perte graduelle des germes survivants les ramène à un niveau numériquement plus bas que celui existant dans le vaccin sec, quand il est conservé à −20° C pendant un an.

3. Les caractères morphologiques, biologiques et de culture des germes survivants le BCG congelé âgé d'un an ne diffèrent pas de ceux du vaccin liquide frais.

4. L'activité antigénique du vaccin BCG glucosé âgé d'un an est plus faible que celle du vaccin liquide frais et le vaccin glucosé congelé âgé d'un an a un pouvoir antigène plus réduit que le vaccin glucosé de même âge; ceci étant démontré par les lésions cutanées, la sensibilité à la tuberculine et la résistance à l'infection provoquée à l'aide de bacilles tuberculeux virulents.

5. En quadruplant le nombre des scarifications cutanées, l'usage de vaccin BCG glucosé sec et de vaccin glucosé congelé produit un degré de résistance à

l'infection provoquée avec des bacilles tuberculeux virulents, qui est comparable à celui induit par le quart du nombre de scarifications cutanées quand on utilise du vaccin liquide frais.

6. Quand il est procédé à une répartition adéquate des scarifications cutanées et du dosage par rapport aux germes vivants ou aux inoculations répétées, l'emploi de vaccin BCG glucosé complétement stabilisé sec ou congelé se montre tout aussi efficace et préférable au vaccin liquide frais, de stabilité incomplète, et dont l'activité antigénique maxima après sa préparation ne dépasse pas 10 jours.

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THE PATHOGENICITY OF STREPTOMYCIN-DEPENDENT TUBERCLE BACILLI[1]

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INTRODUCTION

The isolation of streptomycin-dependent bacteria was first described by Miller and Bohnhoff (1) who recovered a streptomycin-dependent variant of meningococcus by *in vitro* methods and demonstrated that it was pathogenic for mice only when the animals were injected with streptomycin. Since then streptomycin-dependent variants have been isolated from a variety of bacterial species including *Proteus morgani*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium ranae*, and *Mycobacterium tuberculosis* (2–6).

Streptomycin-dependent tubercle bacilli were first isolated from the sputum of a patient receiving streptomycin therapy (4). Guinea pigs were inoculated with streptomycin-dependent cells, and the animals treated with streptomycin had shorter survival times than the untreated animals. The results were interpreted as suggesting enhancement of growth of the streptomycin-dependent cells in the treated animals. It appears unlikely that these studies were performed with a stable[3] streptomycin-dependent variant of tubercle bacilli, since a subsequent report describes the control culture tubes (containing no streptomycin) as showing some visible growth after five days' incubation (7). Also, the *in vitro* results were not consistently reproducible. Streptomycin-dependent tubercle bacilli isolated from mice infected with tubercle bacilli strain H37Rv were reported (5) to have produced disease in mice, regardless of whether or not the animals received streptomycin. The report does not make clear that a stable streptomycin-dependent culture was employed, and the results of the virulence studies are not adequately defined.

Recently we reported a simple technique for the *in vitro* isolation of streptomycin-dependent tubercle bacilli from cultures of *M. tuberculosis* strain H37Rv which had no prior exposure to streptomycin (6). Large populations of tubercle bacilli are composed mainly of streptomycin-sensitive cells. In addition, they include four other types that differ in their relation to streptomycin: (*1*) streptomycin-resistant cells, (*2*) cells which show enhancement of growth in the presence of streptomycin, (*3*) cells which show only an initial growth in the presence of streptomycin, and (*4*) cells which will not grow in the absence of the drug. The purpose of this study was to determine the pathogenicity for guinea pigs

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[3] As used here, "stable" signifies that there is a very low rate of mutation to streptomycin-sensitive or streptomycin-resistant cells even after repeated and prolonged subculturing.

of a stable streptomycin-dependent variant with a low rate of mutation to cells capable of growing in the absence of the drug.

Experimental Procedure

A streptomycin-dependent variant of tubercle bacilli strain H37Rv was isolated and maintained by the technique described previously (6). The cells were subcultured repeatedly in liquid sorbitan mono-oleate albumin medium containing 100 γ of streptomycin per ml., and no growth was observed in the control tubes containing no streptomycin. These bacteria were used in the first experiment (Experiment A).

In the second and third experiments (Experiments B and C), the streptomycin-dependent cells that were used were capable of producing luxuriant growth in the presence of 5 γ of streptomycin per ml. of medium, a concentration readily attained *in vivo*. This strain of tubercle bacilli, which does not grow in the absence of streptomycin, was selected from a culture which originally required 100 γ of the drug per ml. of medium to produce luxuriant growth.

Experiment A: One-milliliter portions[4] of 1:10 dilution of a turbid 10-day-old culture of streptomycin-dependent tubercle bacilli growing in 100 γ of the drug per ml. of medium were inoculated intraperitoneally into each of 10 healthy guinea pigs. Five of these animals were given a 45-day course of streptomycin, 5 mg. subcutaneously twice a day, starting immediately after inoculation with the streptomycin-dependent bacteria; 5 received no treatment. Five additional healthy guinea pigs each received intraperitoneal injections of 1 ml. of 1:10 dilution of a turbid 10-day-old culture of the virulent parent streptomycin-sensitive tubercle bacilli H37Rv. None of these latter 5 animals received streptomycin.

Intracutaneous tuberculin tests, using 5 mg. of Old Tuberculin, were performed at the end of the second week after inoculation and at intervals prior to the conclusion of the experiment.

In the group inoculated with streptomycin-dependent tubercle bacilli, one each of the treated and untreated animals was killed at the end of the fourth and seventh weeks. The remaining 6 animals were killed 18 weeks after inoculation. The organs were examined grossly and microscopically for evidence of tuberculosis. Cultures from the liver, spleen, and other organs were made on plates of solid oleic acid-albumin medium, some with and some without streptomycin incorporated, to demonstrate tubercle bacilli.

Experiment B: Six healthy guinea pigs each received intraperitoneal injections of 1-milliliter portions[4] of a 1:10 dilution of a turbid 10-day-old culture of streptomycin-dependent tubercle bacilli growing in 5 γ of streptomycin per ml. of medium. Twenty-four hours prior to inoculation with the bacteria, 3 of the animals were started on a 45-day course of streptomycin, 5 mg. subcutaneously twice a day. The remaining 3 animals received no streptomycin. Tuberculin tests were done 10 weeks after inoculation, and the animals were killed on the seventy-fifth day. Pathologic and bacteriologic examinations were conducted as in Experiment A.

Experiment C: Because there was minimal histologic evidence of disease in the tissues of the animals inoculated with streptomycin-dependent tubercle bacilli in Experiments A and B, it was decided to conduct a third experiment employing larger doses of the drug and a longer treatment period. Six healthy animals were injected with streptomycin-dependent tubercle bacilli as in Experiment B. Three of the animals were treated with 7.5 mg. of streptomycin subcutaneously twice a day for 100 days. On the one-hundred-first

[4] Each inoculum contained at least 20 million tubercle bacilli.

day the 6 animals were killed and pathologic examinations and cultures were made as in the previous experiments.

RESULTS

Experiment A: The 5 animals infected with the parent tubercle bacilli strain H37Rv died of disseminated tuberculosis within three weeks after inoculation.

Tuberculin tests performed after two weeks on the 10 animals inoculated with streptomycin-dependent tubercle bacilli showed mild erythema and induration but no necrosis. Subsequent tests on the surviving animals showed gradual diminution of the reactions until, at 17 weeks after inoculation, these were only questionable.

The treated and untreated animals killed four weeks after inoculation with streptomycin-dependent tubercle bacilli showed no gross evidence of disease. Cultures made from the spleens of both animals yielded a few colonies on medium containing streptomycin, while no growth was observed in the absence of the drug. The tubercle bacilli recovered from these animals appeared to be of the same variant as that which was injected.

No gross evidence of disease was observed either in the treated and untreated animals killed seven weeks after inoculation or in the remaining 6 animals, 3 of which had received streptomycin, killed after 18 weeks. Cultures of the internal organs were negative for tubercle bacilli. It should be mentioned that all of these animals inoculated with streptomycin-dependent tubercle bacilli appeared well during the course of observation and appeared to be in excellent nutrition at the time of necropsy. The tissues of these animals were examined microscopically and were found to contain exceedingly little evidence of disease.

Experiment B: A very slight reaction to tuberculin was shown after 10 weeks by the 6 animals that had received streptomycin-dependent tubercle bacilli of the strain which required only 5 γ of the drug per ml. of medium. The animals were killed on the seventy-fifth day, and appeared to be in good nutrition. There was no gross evidence of disease and, again, exceedingly few microscopic abnormalities. Cultures of the internal organs of both treated and untreated animals yielded a few colonies in the presence of streptomycin but none in the absence of the drug. The tubercle bacilli appeared to be of the same variant as that which was inoculated.

Experiment C: At autopsy, none of the 6 animals showed any gross evidence of disease. Cultures, both with and without streptomycin in the medium, made from the livers and spleens were negative for tubercle bacilli. The report of the microscopic examinations of the tissues is summarized as follows[4]: (a) Control animals. The lung tissue appears essentially normal; there is some lymphoid hyperplasia but there is no lesion suggesting tuberculous infection. The liver tissue shows lymphocytic infiltration of the portal spaces, and there are scattered necrotic cells with a few associated inflammatory cells in the lobules. Only two of these lesions show recognizable evidence of tuberculosis; one contains a

[4] The microscopic examination of the tissues was performed by Dr. Philip C. Pratt of the Saranac Lake Laboratory of the Trudeau Foundation.

few epithelioid cells; the other, one Langhans' cell. (b) Treated animals. The lung tissue resembles that of the control animals except for the presence of a Langhans' cell in one focus. The liver tissue also resembles that of the controls, having one epithelioid cell focus, although no Langhans' cell is noted. The spleen tissue is not remarkable.

From the above observations it is concluded that the tissues from the control and treated animals are indistinguishable and show exceedingly little evidence of disease.

SUMMARY

The experiments reported indicate that streptomycin-dependent tubercle bacilli isolated by *in vitro* methods from strain H37Rv do not produce progressive disease in guinea pigs. These results are obtained even when the animals are inoculated with large numbers of the bacteria and are treated with streptomycin. In testing the pathogenicity of a streptomycin-dependent variant, the importance of employing a stable culture has been emphasized.

Previous studies (6) have shown that this streptomycin-dependent culture of tubercle bacilli contains approximately 6 streptomycin-resistant cells per 150 million streptomycin-dependent cells. The streptomycin-resistant cells are believed to arise from the streptomycin-dependent cells by a process of mutation. It is assumed that some of the animals in the present experiment may have received one or two of these streptomycin-resistant cells, but apparently the defense mechanisms of the host were sufficient to prevent the development of progressive disease. There was some degree of reaction to tuberculin two weeks after inoculation with the streptomycin-dependent bacteria but subsequent tuberculin tests showed progressive diminution in the intensity of the reactions. Although it was possible to isolate a few streptomycin-dependent tubercle bacilli from animals killed four and ten weeks after inoculation, there is little evidence that any appreciable multiplication of the cells occurred *in vivo*.

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STUDIES IN THE METABOLISM OF THE TUBERCLE BACILLUS[1]

II. The Production of a Pharmacologically Active Metabolite, with a Note on the Utilization of Histidine

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INTRODUCTION

While studying the possibility of degradation of histidine to histamine by the tubercle bacillus grown on synthetic media, and while assaying culture filtrates for histamine by the method of utilizing the isolated guinea pig or rabbit ileum, it was observed that culture filtrates to which no histidine had been added often produced a characteristic depressor effect on this isolated tissue. More constant and more pronounced effects were produced by such culture filtrates on the isolated ileum of the rabbit. The latter test object was, therefore, employed in an attempt to elucidate the chemical nature of the metabolite and its possible significance.

The immediate question at hand, viz., the metabolic degradation of histidine to histamine by tubercle bacilli when the amino acid was added to Kirchner's medium, was soon answered in the negative. Assays by the isolated ileum of the guinea pig for histidine and histamine in such culture filtrates, with their appropriate controls, failed to reveal formation of histamine although there was definite evidence of appreciable loss of histidine from the medium during the course of growth of the tubercle bacillus. The results of a typical experiment are illustrated in figure 1. These experiments appeared to confirm the earlier work by Hanke and Koessler (1) who arrived at similar conclusions based on colorimetric tests, and are consistent with Campbell's suggestion that histidine is not decarboxylated by the tubercle bacillus but that it may be deaminated and subsequently oxidized to imidazole acetic acid (2).

PHARMACOLOGIC PROPERTIES OF THE METABOLITE

Primary depression, occasionally followed by marked secondary stimulation, was the effect produced on the isolated ileum of the rabbit by 0.5 to 3 cc. of culture filtrates of tubercle bacilli added to 100 cc. of oxygenated Ringer-Locke solution bathing the tissue. The culture filtrates were obtained after autoclaving 3 to 8 weeks' growth of tubercle bacilli on Kirchner's medium[3] incubated at 38°C. The degree of depression produced was related to the dose of filtrate and varied from a transient reduction in amplitude to complete abolition of the normal spontaneous contractions (figure 2).

A considerable number of pathogenic strains of tubercle bacilli, human and

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[2] Fellow of the National Institutes of Health.

[3] For the composition of this medium see reference (4).

bovine, were used in this work and all gave similar results[4]. Two avirulent strains, BCG and *Mycobacterium* 607, were tested and also gave the same results (figure 2).[5]

The constancy of the reaction elicited by culture filtrates of diverse strains of acid-fast organisms and the quantitative aspects of the reaction as related to dose suggested that we were dealing with a definite chemical individual. Various substances known or suspected to be produced by growing tubercle bacilli were tested, with negative results. These substances included PPD, 8 mg.; riboflavin, 0.1 mg.; *p*-aminobenzoic acid in amounts as large as 100 mg.; soluble vitamin K,

FIG. 1. Failure of the tubercle bacillus to metabolize histidine to histamine. Guinea pig ileum in 100 cc. of oxygenated Ringer-Locke solution at 38°C. Time in minutes.

1—3. 0.1, 1.0, and 8 mg. of histidine, respectively.

4. 0.5 γ of histamine hydrochloride.

5. 0.4 cc. of control medium containing 8 mg. of histidine incubated 19 days.

6. 0.01 cc. of H37Rv filtrate with 0.2 mg. of histidine incubated 19 days.

7. 0.05 cc. of same filtrate as above with 1 mg. of histidine incubated 19 days. Shows disappearance and probable utilization of histidine without formation of histamine.

30 mg.; saturated solution of phthiocol, 3 cc.; inositol, 100 mg.; nicotinic acid and nicotinamide, each 50 mg.; calcium pantothenate, 3 mg.; and folic acid, 1.6 mg. The above were added to 100 cc. of Ringer-Locke solution in the amounts indicated. In no instance was a reaction elicited in any way resembling that of the metabolite under consideration.

Examination of several of the nucleic acid degradation products revealed that adenylic acid[6] mimicked the pharmacologic effects of the culture filtrates in all essential details. The similarity of effects was so close that it seemed justifiable to use adenylic acid as a standard of reference in assaying culture filtrates for

[4] Human: H37Rv, A27, A31, 7690, A32, 279, 7989; bovine: Ravenel.

[5] There is no satisfactory explanation at this time for the occasional biphasic reaction produced by some culture filtrates. It is possible that some of the filtrates may have another metabolite, such as choline, in small amounts.

[6] Adenosine-3-phosphoric acid.

their metabolite content under varying experimental conditions. Absorption studies in the ultraviolet region of the spectrum gave a maximum absorption for the culture filtrates when properly diluted (1:20) at 260 mu, the same as for adenylic acid dissolved in Kirchner's medium in concentrations of 5 to 20 γ per

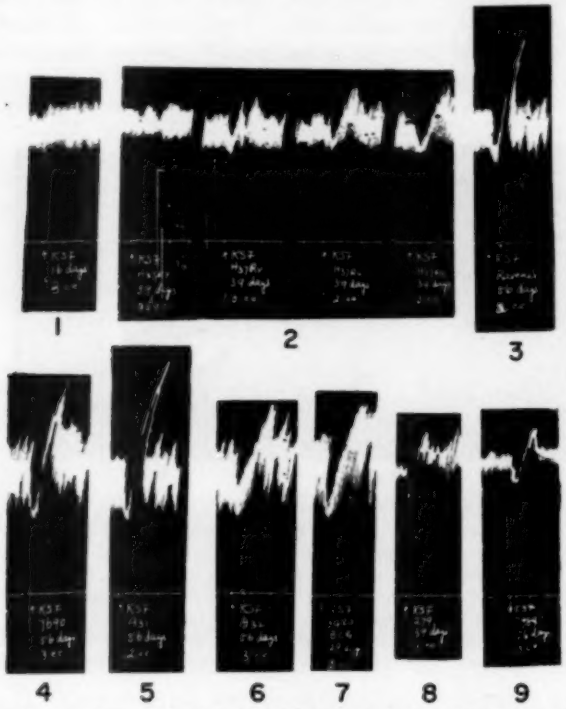

FIG. 2. Production of metabolite by eight different strains of acid-fast organisms. Rabbi ileum in 100 cc. of oxygenated Ringer-Locke solution at 38°C.

1. 3 cc. of control medium incubated 16 days.
2. Effect of graded doses, 0.5–3.0 cc., of culture filtrate of H37Rv incubated 39 days.
3. 2 cc. of culture filtrate of bovine Ravenel strain incubated 56 days.
4—9. Effects of 2–3 cc. amounts of culture filtrates of 5 human strains and one BCG strain incubated 29–56 days.

cc. (table 1). Of the many nucleic acid derivatives tested, only adenosine and adenosine triphosphate had an activity comparable with adenylic acid. Ribonucleic acid and desoxyribonucleic acid had slight or no activity. The following nucleic acid derivatives were inactive: guanine, guanosine, guanylic acid, cytidine, cytidylic acid, uridine, uridylic acid, thymine, and uracil. Adenylic acid was chosen as the standard of reference in all the assay work, as illustrated in figures 3 and 4.

CONCENTRATION OF THE METABOLITE IN CULTURE FILTRATES

It was of interest to learn whether the concentration of the metabolite in culture filtrates could be influenced by changes in the nutrients in the medium, length of incubation, or other factors. The production of riboflavin has been

TABLE 1

Comparison of Ultraviolet Absorption at pH 7.0 of Adenosine-3-Phosphoric Acid and Culture Filtrate

MU	OPTICAL DENSITY	
	Adenosine-3-Phosphoric acid, 10 γ/cc.	Culture filtrate 1:20
300	0	0.088
290	0.004	0.152
280	0.065	0.288
270	0.300	0.442
265	0.415	0.490
260	0.456	0.496
255	0.422	0.456
250	0.342	0.378
240	0.152	0.165
230	0.079	—
220	0.169	—

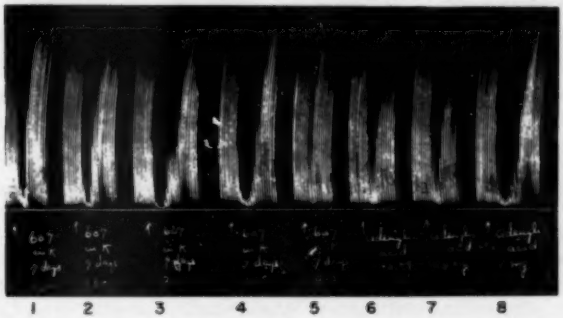

FIG. 3. Production of metabolite by *Mycobacterium* 607 and typical assay against adenylic acid as standard. Rabbit ileum.

0.25 cc. of filtrate (7 days' incubation) approximately equals in activity 0.25 mg. of adenylic acid.

found to be influenced by the quality and quantity of the nitrogen in the medium (3) as well as by the incubation period, the strain of organisms, and the presence or absence of riboflavin precursors (4). It may be supposed that the metabolite may exist partly in combination with riboflavin as a dinucleotide. If so, the amount of metabolite present at any one time might be related to the amount of riboflavin in the system.

A series of culture filtrates obtained under conditions designed to produce concentrations of riboflavin in the range of 61 to 284 γ per 100 cc. were assayed for the metabolite, using adenylic acid as a reference standard. These assays yielded values of 23 to 45 mg. per 100 cc., with an average of 32 mg. per 100 cc. It would appear, therefore, that the adenylic acid-like metabolite is produced at a more uniform rate, independent of riboflavin, and is not readily influenced by changes in experimental conditions.

PRODUCTION OF THE METABOLITE BY STREPTOMYCIN-RESISTANT STRAINS

Two strains of streptomycin-resistant organisms were used in this work. One was a resistant variant of H37Rv which grows in 1,000 γ of streptomycin per

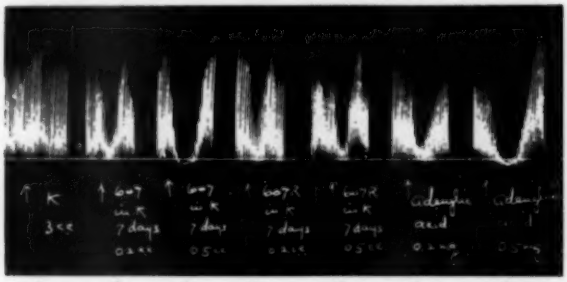

FIG. 4. Production of metabolite by *Mycobacterium* 607, streptomycin-sensitive and streptomycin-resistant variant. Assay with adenylic acid as standard. Rabbit ileum.

1. 3 cc. of control medium incubated 7 days.

2-3. 0.2 and 0.5 cc., respectively, of culture filtrate of streptomycin-sensitive strain incubated 7 days.

4-5. 0.2 and 0.5 cc. as above, streptomycin-resistant variant.

6-7. 0.2 and 0.5 mg., respectively, of adenylic acid.

Approximately 100 mg. per 100 cc. adenylic acid equivalent produced by both strains.

cc.[7] The other was a variant of *Mycobacterium* 607 resistant to 40,000 γ of streptomycin per cc. The latter was secured in this laboratory by a single subculture through the simple expedient of adding streptomycin to a young culture of nearly full growth, with subsequent further incubation for several days, followed by subculture. *Mycobacterium* 607 grows rapidly, and two to three days after inoculation of 50 cc. of Kirchner's medium in a 125 cc. flask approximately three-quarters of the surface is covered with the growth. At this time a sterile solution of streptomycin is added to make a concentration of 200 γ per cc. The flask is shaken lightly and returned to the incubator for another 5 to 11 days to permit the multiplication of the relatively few resistant organisms presumably originally present in the culture. The old growth drops to the bottom of the flask and a small new growth is formed, partially covering the surface. A pellicle of this planted on Kirchner's medium will grow well in the presence of as much as 4 per

[7] Kindly supplied by Mr. W. Steenken, Jr., of the Trudeau Laboratory.

cent of streptomycin. Indeed, this variant has been found to grow in a modified Kirchner's medium with streptomycin as the only source of nitrogen.

Culture filtrates of the two streptomycin-resistant strains described above were tested for metabolite content. The results showed no significant deviation from that produced by the parent strains under similar experimental conditions. This is shown in figures 4 and 5.

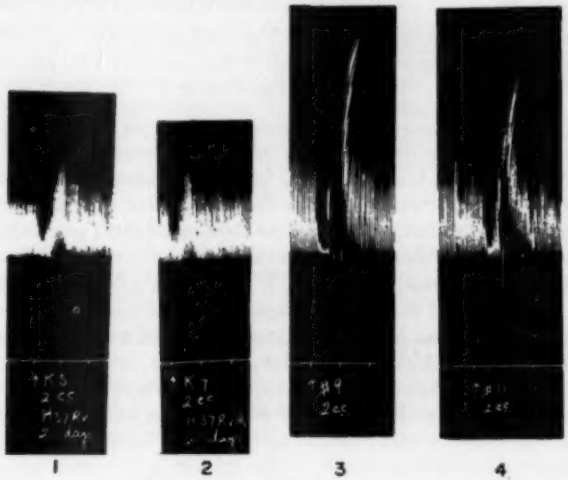

FIG. 5. Production of metabolite by streptomycin-resistant variant of H37RV. Rabbit ileum.

1–2. 2 cc. each of culture filtrates of resistant and parent strains, incubated 21 days.

3–4. 2 cc. each as above, incubated 36 days.

SUMMARY AND CONCLUSIONS

In a study of the metabolic degradation of histidine to histamine by tubercle bacilli grown on the surface of synthetic liquid (Kirchner's) medium, a hitherto undescribed pharmacologically active metabolite was demonstrated in the culture filtrates of a variety of acid-fast microorganisms. The metabolite was found to be produced by human and bovine tubercle bacilli, pathogenic and nonpathogenic alike. Pharmacologically, the metabolite is characterized principally by a depressant action on the isolated ileum of the rabbit. In some instances, the depressant action is followed by stimulation. It resembles the action of adenylic acid and can be assayed with the latter substance as a reference standard. The absorption spectrum of the metabolite in the ultraviolet region is the same as that of adenylic acid.

The production of the metabolite by tubercle bacilli on media that support normal growth is fairly uniform, and not readily influenced by experimental conditions capable of affecting riboflavin production. Streptomycin-resistant organisms produced the metabolite at the same rate as the parent strains.

No evidence could be obtained of the formation of histamine from histidine added to the culture medium even though the partial disappearance of histidine from the medium could be demonstrated, presumably indicative of its utilization by the growing tubercle bacillus. It is inferred from this that histidine is not decarboxylated by the tubercle bacillus, but may be degraded by deamination.

SUMARIO Y CONCLUSIONES

Estudios del Metabolismo del Bacilo Tuberculoso: II. La Producción de un Metabolito Farmacológicamente Activo; con una Nota sobre la Utilización de la Histidina

Mientras se estudiaba la degradación metabólica de histidina en histamina por el bacilo tuberculoso cultivado en la superficie de un medio líquido sintético (el de Kirchner), se descubrió un metabolito farmacológicamente activo, no descrito hasta ahora, en los filtrados culturales de varios microbios ácidorresistentes. Los bacilos tuberculosos humanos y bovinos, tanto patógenos cuanto anatógenos, lo producían. Farmacológicamente, caracterízase el metabolito principalmente por su efecto depresor, seguido a veces de excitación, sobre el ileon aislado del conejo. Su acción semeja la del ácido adenílico, con el cual, como tipo de comparación, puede valorarse. El espectro de absorción del metabolito en la región ultravioleta es idéntico al de dicho ácido.

La producción del metabolito por bacilos tuberculosos en medios que sostienen la proliferación normal es bastante uniforme, sin que la afecten fácilmente los factores experimentales susceptibles de afectar la producción de riboflavina. Los microbios estreptomicinorresistentes lo produjeron a la misma velocidad que las cepas matrices.

No pudieron obtenerse pruebas de la formación de histamina, de la histidina agregada al medio de cultivo, aunque pudo observarse la desaparición parcial de la última, del medio, presunta indicación esta de la utilización de la histidina por el bacilo tuberculoso en vías de crecimiento. Infiérese de lo anterior que la histidina no es decarboxilada por el bacilo tuberculoso, pero puede ser degradada por deaminación.

RESUME ET CONCLUSIONS

Etudes sur le métabolisme du Bacille tuberculeux: II. Production d'un métabolite pharmacologiquement actif et une note sur l'utilisation de l'histidine

Au cours de l'étude de la dégradation métabolique de l'histidine en histamine par le bacille tuberculeux cultivé à la surface du milieu synthétique de Kirchner, un métabolite doué de propriétés pharmacologiques, et non encore décrit jusqu'à présent, a été découvert dans le filtrat de cultures de divers germes acido-résistants. Il a été produit par les bacilles tuberculeux humains et bovins, aussi bien pathogènes que non pathogènes. Du point de vue pharmacologique ce métabolite est caractérisé principalement par une action dépressive sur l'iléon du lapin, à laquelle succède parfois une action de stimulation. Son action est comparable à celle de l'acide adénylique qui peut servir d'étalon pour mesurer son

activité. Le spectre d'absorption de ce métabolite au niveau de l'ultra-violet est le même que celui de l'acide adénylique.

La formation du métabolite par le bacille tuberculeux cultivé dans un milieu favorable à son développement normal est sensiblement uniforme et n'est pas facilement influencée par les conditions expérimentales capables d'affecter la production de riboflavine. Il a été formé par des germes streptomycino-résistants dans la même proportion que par les souches-mères.

La formation d'histamine dérivée de l'histidine ajoutée au milieu de culture n'a pas été observée malgré que la disparition partielle de l'histidine du milieu ait été constatée, ce qui semble indiquer son utilisation par les bacilles en coure de développement. La conclusion de ceci est que l'histidine n'est pas soumise à la décarboxylation par le bacille tuberculeux, mais qu'elle pourrait résulter d'une déamination.

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HYALURONIDASE IN TUBERCULOSIS[1,2]

H. J. CORPER AND MAURICE L. COHN

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INTRODUCTION

The enzyme hyaluronidase is a nontoxic, nonantigenic substance which aids the absorption of colloids and crystalloids, the spreading of particulate materials, and which enhances the diffusion and thus the speed of action of a variety of therapeutic agents (1–4). The availability of hyaluronidase[3] of established unit potency (turbidity-reducing units) (5) made it possible to study more accurately the effects of a specific spreading agent upon infections and especially on tuberculosis. This report presents the results of an investigation of the effect of hyaluronidase on the local intracutaneous spread of tuberculosis from the site of inoculation. In order to control this adequately, the injections with and without hyaluronidase had to be introduced into the skin of the same animal at different sites, thus making the study of the effect upon the generalized spread of the disease of little value in these animals, although the local effect proved to be striking. The observations included, insofar as possible, a study of the tributary lymph nodes.

Pertinent to the problem at hand are the observations of Hechter (6) who noted that the effect of hyaluronidase in reduction of the tissue barrier persists for approximately forty-eight hours, the maximum effects occurring shortly after introduction. Elster and Lowry (7) found that maximum concentration of the enzyme was attained in rats within five minutes after intravenous injection and thereafter the concentration diminished rapidly so that it was no longer detectable by turbidimetric methods after one hour.

EXPERIMENTAL

Intracutaneous injection: Virulent human tubercle bacilli (0.001 mg. in 0.1 cc.) were injected intracutaneously into guinea pigs as follows: (a) together with hyaluronidase, 5 turbidity units; (b) injected at a site into which hyaluronidase, 5 turbidity units, was injected one hour later; (c) bacilli injected alone (controls). The results of this experiment are presented in figure 1.

In considering the significance of these data, it is well to remember that, as demonstrated in 1936 (8), in the intracutaneous injection of particulate materials (india ink), the primary and important determinant of the area of deposition is the concentration of the material. Secondary to this, but influencing it, is the

[1] From the Research Department, National Jewish Hospital at Denver, and the University of Colorado School of Medicine.

[2] This investigation was aided by a gift from Morton May in memory of Florence G. May.

[3] The hyaluronidase used in these studies was the carefully titrated "Hyronase" (Hyaluronidase-Schering) supplied as 5 to 150 turbidity-reducing units (see reference (5)) by the Schering Corporation of Bloomfield, New Jersey.

fact that the higher the concentration, the greater the chance for lymphatic extension even to the point of general systemic distribution along certain definite channels. In addition, a definite filtration concentration of the particulate material occurred at the needle point of inoculation. The volume of the injection played only a small part in distribution.

The data in figure 1 indicate that 5 turbidity-reducing units of hyaluronidase injected coincident with, or one hour after, the intracutaneous injection of virulent human tubercle bacilli (0.001 mg.) exerted a marked spreading effect upon the bacillary suspension locally, and produced multiple skin tubercles as well as more extensive ulceration. Although not illustrated in the figure, 5 tur-

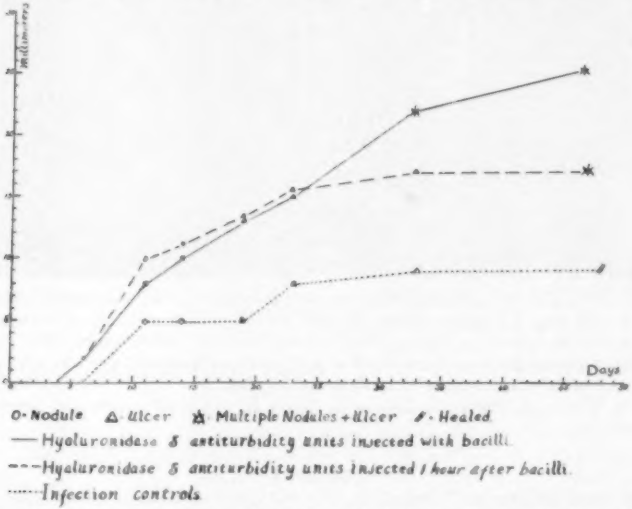
Days
O-Nodule △-Ulcer ☆-Multiple Nodules+Ulcer ✓-Healed
—Hyaluronidase 5 antiturbidity units injected with bacilli
---Hyaluronidase 5 antiturbidity units injected 1 hour after bacilli
·····Infection controls

FIG. 1. The development of the tuberculous skin lesions following the intracutaneous injection of virulent human tubercle bacilli with and without hyaluronidase.

bidity-reducing units of hyaluronidase given one hour prior to the bacillary suspension produced a slight but definite enhancing effect upon the local tuberculosis.

Table 1 presents observations in the same animals (guinea pigs) on the local tributary glandular involvement and the generalized disease at autopsy 77 days after infection.

It appears evident from table 1 that, although a more widespread local skin tuberculosis resulted from the injection of hyaluronidase with the bacilli, the local tributary glandular tuberculosis was less on the side of the more marked skin involvement. Although interpretation is difficult, the factors involved appear related to the well-known phenomenon in which large local lesions of slow development frequently bring into play the retarding effect of a rising specific immunity, which is less evident with lesser local effects.

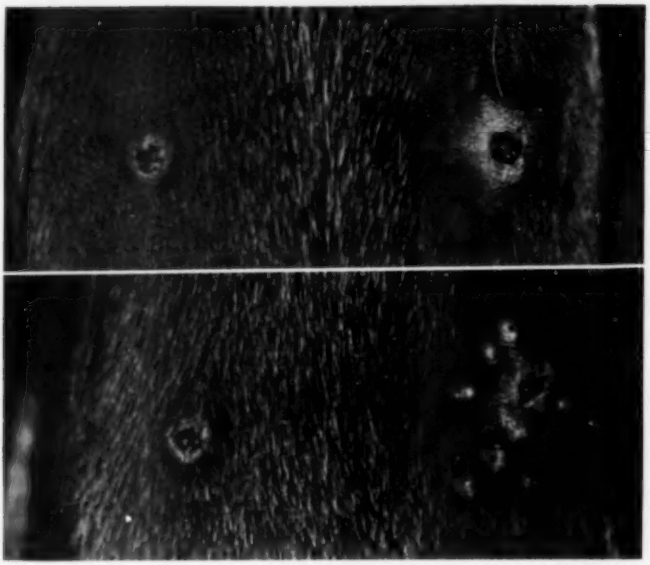

FIG. 2. (Top) The effect of coincident injection of 5 turbidity-reducing units of hyaluronidase and 0.001 mg. of virulent human tubercle bacilli, *S* 4008, upon the skin tuberculosis three weeks after infection. Note marked increase in size of hyaluronidase site (on right) with multiple small peripheral tubercles as compared with control site (on left) injected with same bacillary suspension without hyaluronidase.

FIG. 3. (Bottom) The same lesions as figure 2, five weeks after the intracutaneous injections.

TABLE 1

Effect of Hyaluronidase on Tuberculosis following Intracutaneous Infection with Virulent Human Tubercle Bacilli (S 4908)

TIME OF ADMINISTRATION OF HYALURONIDASE* IN RELATION TO INJECTION OF TUBERCLE BACILLI	AVERAGE WEIGHT OF INGUINAL GLANDS (GRAMS)		TUBERCULOSIS 77 DAYS AFTER INFECTION
	Control Right side of abdomen	Hyaluronidase Left side of abdomen	
Coincident	2.4	1.8	1, 2, 1, 1, 1†
1 hour prior to infection	5.9	3.1	3, 3, 1, 2, 1
1 hour after bacillary infection	2.2	1.8	3, 3, 2, 2, 2

* Hyronase, Schering Corporation brand was used.

† These numerals present the arbitrary grading from 0 = no visible tuberculosis to 4 = a generalized massive infection of the amount of tuberculosis found at autopsy.

Subcutaneous injection: It seemed important to note the effects of systemic administration of small and large amounts of hyaluronidase on the development

of generalized tuberculosis. This also appeared important because of the work of Berquist and Packalen (9), who noted a diminution in the life span of tuberculous guinea pigs, from an average of 71 days to 53 days, after the administration of *Staphylococci*, which are known to produce hyaluronidase. The latter experiments, however, were performed with relatively large infecting amounts of tubercle bacilli, 0.5 cc. of a 5-day culture of H37 in Tween-albumin medium.

TABLE 2

Effect of Hyaluronidase on the Spread of Virulent Human Tubercle Bacilli from the Subcutaneous Infection Site

TIME OF ADMINISTRATION OF HYALURONIDASE* IN RELATION TO INJECTION OF TUBERCLE BACILLI	TUBERCULOSIS 75 DAYS AFTER INFECTION†	AVERAGE TUBERCULOSIS
None	3, 3, 3, 3, 3	3
1 hour before	3, 4, 4, 4, 3	4
Coincident	3, 3, 3, 3, 2	3
1 hour after	4, 3, 4, 4, 3	4

* Hyronase, Schering Corporation brand in 5 turbidity-reducing units used.
† 0.0001 mg. virulent human tubercle bacilli (#4008) was used for infection.

TABLE 3

The Effect of Repeatedly Administered Hyaluronidase on Tuberculosis

INFECTION	DOSE OF HYALURONIDASE (TURBIDITY REDUCING UNITS)	AVERAGE WEIGHT OF INGUINAL GLANDS	TUBERCULOSIS 60 DAYS AFTER INFECTION
		gm.	
0.0001 mg. #4008, subcutaneously	None; control	0.7	3, 3, 3†
	25 U daily for 30 days*	1.8	3, 3, 3
0.001 mg. #4008, intravenously	None; control	0.7	3, 4, 4
	25 U daily for 30 days	1.0	3, 3, 3

* The hyaluronidase was injected subcutaneously on the side opposite infection site.
† The amount of tuberculosis found in the guinea pigs 60 days after infection is recorded arbitrarily by numerals from 0 = no evident tuberculosis to 4 = a massive generalized disease involving all the important organs.

In table 2 are given the results of the subcutaneous infection with 0.0001 mg. of virulent human tubercle bacilli (#4008) and subcutaneous injection of hyaluronidase, 5 turbidity-reducing units, into the site in single injection coincidently, one hour before, and one hour after, the infection.

It is evident from table 2 that an amount of hyaluronidase, 5 turbidity-reducing units, capable of enhancing an intracutaneous skin tuberculosis, was incapable of influencing appreciably the tuberculosis resulting from subcutaneous infection.

Repeated injections: In order to decide whether repeated large doses of hyaluronidase might influence the general tuberculosis resulting from subcutaneous

or intravenous infection, 25 turbidity-reducing units of hyaluronidase were given subcutaneously to guinea pigs daily for 30 days from the initiation of infection (0.0001 mg. subcutaneously or 0.001 mg. intravenously of virulent human tubercle bacilli, # 4008). The results of this study are recorded in table 3.

It is evident from the data in table 3 that 25 turbidity-reducing units of hyaluronidase given daily subcutaneously for 30 days exert no appreciable effect upon the tuberculosis in guinea pigs produced by either subcutaneous or intravenous infection, although a slightly greater glandular hypertrophy was noted in the hyaluronidase-treated guinea pigs as compared with controls infected only. Pertinent to these results is the fact that Banks, Seligman, and Fine (4) noted no effect of hyaluronidase upon the absorption of parenterally administered radioactive plasma proteins in the dog following its injection intraperitoneally, although subcutaneous absorption was enhanced 1.5 to 3 times.

DISCUSSION

Hyaluronidase has been recommended variously for speeding penetration into disease foci as well as into microorganisms causing the disease. Indeed the effects of spreading agents are best known in the field of pathology. All infectious agents which are pathogenic for, or propagated through, the connective tissue when injected in adequate amounts have enhanced local primary effects in tissues permeated by the spreading factor from any source. In general, organisms characterized by a capacity for dramatic invasion (like *Staphylococci* and certain *Streptococci*) produce spreading factors. In contrast to invasive species, many others are devoid of spreading factors, among which are included all types of tubercle bacilli. Walker and Hoffman (11) noted an enhancing effect of testicular (rabbit) extract on human, bovine, and avian cutaneous tuberculosis in a limited experiment, but were apparently unaware of the nature of the reaction or its mechanism. Berquist and Packalen (9) demonstrated that the extension of an experimentally induced local tuberculous infection in the skin of rabbits or guinea pigs was increased by the simultaneous injection of hyaluronidase-producing bacteria such as *Staphylococci* and *Streptococci*. A direct correlation was claimed between the enhancing effect of the accompanying bacteria and their capacity to produce hyaluronidase. In many of the experiments, the spreading effect appeared to be caused, in part at least, by enzymes preformed in the bacterial culture. Other experiments, however, showed clearly that spread had occurred because hyaluronidase was produced at the site of infection. The generalized tuberculous infection in the skin of guinea pigs simultaneously inoculated with tubercle bacilli and hyaluronidase-producing *Staphylococci* was apparently aggravated and accelerated in proportion to the enzyme production of the strain used. Naturally the inducement is great to use a spreading agent to enhance absorption of therapeutic materials, especially since a commercial preparation of a standardized hyaluronidase is in contemplation. Such use may be justified under certain conditions to enhance general as well as noninfectious local therapeutic effects; yet in the presence of infectious agents at the site of injection

caution should be exercised, especially with a disease like tuberculosis where misinterpreted apparent early benefit may be actually harmful.

SUMMARY

An investigation has been made of the effects of the enzyme hyaluronidase on the spread of tuberculosis in guinea pigs infected by injection of tubercle bacilli intracutaneously, subcutaneously, and intravenously. It was found that hyaluronidase injected intracutaneously at the site of intracutaneous injection of tubercle bacilli, together with, or one hour after, injection of the bacilli exerts a marked spreading effect on the bacillary suspension, resulting in the development of multiple skin tubercles as well as more extensive ulceration.

Subcutaneous injection of hyaluronidase, 25 units daily for 30 days in guinea pigs, exerted no appreciable effect on the generalized tuberculosis resulting from subcutaneous or intravenous injection of virulent human tubercle bacilli. These experiments corroborate earlier observations on spreading effects on tuberculosis of less pure agents, such as testicular extracts, *Staphylococci*, and *Streptococci*, which contain or form hyaluronidase.

It is conceivable that hyaluronidase can be used efficiently under certain circumstances to aid penetration of therapeutic agents into disease foci with beneficial results. It does not seem advisable, however, to use hyaluronidase to speed entrance of therapeutic agents into disease foci, such as those in tuberculosis, when these agents are incapable of destroying the bacilli readily. (For example, streptomycin, even 1,000 γ per cc. in contact *in vitro* for three months, does not destroy the viability of tubercle bacilli.) Thus, local spread of the bacilli and disease foci may result, as occurred in the experiments recorded here.

SUMARIO

La Hialuronidasa en la Tuberculosis

Por las investigaciones realizadas con la hialuronidasa, el elemento dispersador de encimas y por el efecto de la misma sobre la intensificación de la tuberculosis local, resulta evidente que puede producir efectos contraproducentes si se aplica directamente a una zona en que haya bacilos tuberculosos presentes.

Estos experimentos corroboran observaciones anteriores acerca del efecto difusor ejercido sobre la tuberculosis por sustancias menos puras, tales como extractos testiculares, estafilococos y estreptococos que contienen y forman hialuronidasa.

Es concebible que pueda usarse eficazmente la hialuronidasa en ciertas circunstancias para fomentar la penetración de agentes terapéuticos en focos patógenos con resultado beneficioso, máxime si destruye el agente infeccioso a la par que acelera la absorción de agentes dotados de acción general. Sin embargo, no parece prudente emplear la hialuronidasa para acelerar la entrada de ciertos antibióticos u otros agentes terapéuticos en focos patógenos, como los tuberculosos, si esos antibióticos o agentes (v. g., estreptomicina—ni 1,000 γ por cc. en contacto *in vitro* durante tres meses destruyen la viabilidad de los bacilos tubercu-

losos) son incapaces de destruir fácilmente dichos bacilos. La razón es que sólo se logra así una difusión local de los bacilos y focos, según sucedió en los experimentos aquí presentados, con el correspondiente efecto adverso.

RESUME

Hyaluronidase dans la tuberculose

A la suite des recherches faites avec l'hyaluronidase, enzyme facteur de diffusion ("spreading agent") et qui sont rapportées ici, il est évident que, en raison de son action favorisant le développement des foyers tuberculeux, cette substance peut avoir une action défavorable quand elle est appliquée directement sur une région où le bacille tuberculeux est présent.

Ces recherches expérimentales corroborent les observations antérieures sur l'effet de dissémination de la tuberculose produit par des agents de moindre pureté, tels qu'extraits testiculaires, staphylocoques ou streptocoques contenant et produisant de l'hyaluronidase.

Il est admissible que l'hyaluronidase puisse être utilisée favorablement dans certaines circonstances pour favoriser la pénétration d'agents thérapeutiques dans les foyers pathologiques, en particulier quand elle détruit l'agent infectant en même temps qu'elle active l'absorption d'agents ayant une action générale. Toutefois, il ne paraît pas recommandable de se servir d'hyaluronidase pour activer l'introduction de certains antibiotiques ou autres agents thérapeutiques dans des foyers d'infection, tels que les foyers tuberculeux, quand ces agents ou antibiotiques (par ex. la streptomycine à la concentration de 1.000 γ par cm³ laissée en contact avec le bacille tuberculeux, *in vitro*, pendant trois mois ne détruit pas sa viabilité) n'ont pas le pouvoir de détruire rapidement ces bacilles. C'est ainsi qu'au cours de l'expérimentation relatée ici il s'est produit une diffusion du bacille et l'extension du foyer d'infection, d'où résultat défavorable correspondant.

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Case Report

INTRAPERITONEAL HEMORRHAGE OCCURRING AS A COMPLICATION OF PNEUMOPERITONEUM[1,2]

JOHN C. LARKIN, JR., AND SAMUEL PHILLIPS

(Received for publication July 14, 1950)

INTRODUCTION

Therapeutic pneumoperitoneum is reported to be a procedure with few complications. Mitchell and his associates (1), however, have cited the following to be the more commonly reported complications: peritoneal effusion and peritonitis, aggravation of various types of abdominal hernias, subcutaneous emphysema, dyspnea, and abdominal pain. Less frequent complications are adhesive peritonitis, an increased incidence of acute appendicitis, mediastinal emphysema, accidental pneumothorax, aggravation of pre-existing heart disease, increased hemoptysis, rupture of the diaphragm, atrophy of the diaphragm, dysmenorrhea, aggravation of pre-existing enteritis, hemorrhage from the rectum, "peritoneal shock," massive atelectasis, lobular atelectasis, and air embolism.

In a report which lists the complications of pneumoperitoneum of sufficient severity to require cessation of pneumoperitoneum therapy in 7 per cent of a group of 407 patients, Trimble and his associates (2) mention the occurrence in one patient of hemorrhage within the spleen requiring surgical repair. This case is not described in detail. Habeeb and Reiser (3), in a discussion of the complications of pneumoperitoneum, stated that penetration of a hollow viscus by the needle had not been followed by additional complications. They mentioned a report of puncture of the inferior epigastric artery, but did not describe the case.

The present report is concerned with a patient who developed intraperitoneal hemorrhage as a complication of pneumoperitoneum, and it is believed that no similar case has heretofore been reported.

CASE REPORT

J. D., a 28-year-old white male, entered Kennedy Veterans Hospital May 16, 1950 to have a thoracoplasty for closure of a tuberculous cavity in the apex of the left lung. The diagnosis of pulmonary tuberculosis was first made in 1943. A left pneumothorax was then attempted but was unsuccessful because of adhesions. In 1945 a left phrenic crush was attempted. A left phrenicectomy was performed in 1947 and pneumoperitoneum therapy instituted. He had continued to receive weekly refills of approximately 1,000 cc. of air up to the time of present hospitalization. In December 1949 he was re-examined at another Veterans Administration hospital where a cavity was discovered in the left upper lobe and cultures of several gastric aspirates were found to be positive for tubercle bacilli. A thoracoplasty on the left was advised but was refused by the patient. He later changed his mind and was admitted

[1] From the Tuberculosis Section, Medical Service, the Veterans Administration Medical Teaching Group, Kennedy Hospital, Memphis, Tennessee.

[2] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

to Kennedy Hospital for the procedure. At the time of admission he had a mild, intermittent cough productive of approximately one-half ounce of mucoid sputum. There had been no fever, night sweats, hemoptysis, or weight loss.

Physical examination revealed a well-developed, well-nourished, white male. The eyes, ears, nose, and throat were normal. The phrenicectomy scar was present. The lungs were clear to percussion and auscultation. The blood pressure was 120 systolic and 90 diastolic. No cardiac enlargement or murmurs were present. The abdomen was soft, and the signs of pneumoperitoneum were present. No tenderness or enlarged organs were noted in the abdomen. The genitalia, rectum, and extremities were normal except for a small bony protuberance above the lateral epicondyle of the left humerus.

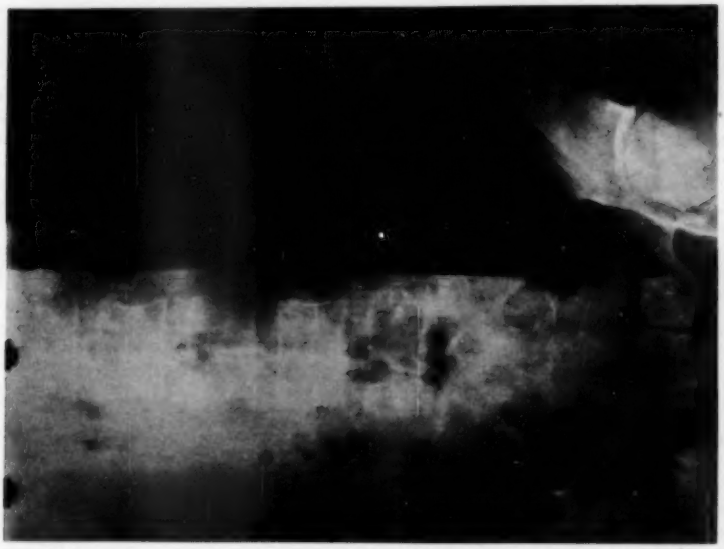

FIG. 1. Roentgenogram of the abdomen in the lateral decubitus position revealing a fluid level.

Complete blood count and urinalysis were normal. Microscopic examination of a sputum smear was positive for acid-fast bacilli. A chest roentgenogram showed a pneumoperitoneum and fibrocalcific densities in both upper lung fields, with no definite area of cavitation. A laminagram, however, revealed a triangular area of radiolucency in the left upper lung field.

On June 6, the patient received his weekly pneumoperitoneum refill in preparation for the contemplated surgery. The initial pressure was +4 cm. of water, and 1,000 cc. of air were instilled without difficulty. The final pressure was +8 cm. of water. Following the refill, the patient stated that he felt a slight discomfort. That evening after lying quietly in bed, he turned on his left side and almost immediately experienced a mild "tearing" sensation in the left upper quadrant of the abdomen. Subsequently, he began to notice abdominal pain and weakness. That night his blood pressure was 120 systolic and 70 diastolic, pulse rate 72 per minute, and temperature 98.4° F. There was slight tenderness at the site of the pneumoperitoneum refill but no rigidity. No blood was present in the stool.

The abdominal pain was still present on the following day, along with anorexia, but there was no fever or abdominal rigidity. The erythrocyte count had fallen from 4.7 million per cu. mm. on admission to 3.7 million, and the concentration of hemoglobin from 14.4 to 11.1 gm. per 100 cc.

It was believed that the patient probably had an intraperitoneal hemorrhage most likely due to bleeding from a ruptured adhesion. He was observed closely, with the thought that if there were evidence of continued bleeding he would require a laparotomy. The following morning he was quite pale and weak and continued to complain of abdominal pain. There was also a feeling of soreness in both shoulders. He had vomited a small amount of watery material. His blood pressure was 92 systolic and 70 diastolic, and temperature, 98.2° F. There was slight abdominal tenderness but no rigidity. On rectal examination, no tenderness or masses were noted and the feces were normal in color. Peristaltic sounds appeared normal. The erythrocyte count had fallen to 2.7 million per cu. mm. and the hemoglobin concentration to 8 gm. per 100 cc. A roentgenogram of the abdomen in the lateral decubitus position revealed a fluid level (figure 1). During the day the patient received 1,500 cc. of whole blood by transfusion and felt greatly improved, with a diminution of weakness and abdominal pain. But that night there was a return of weakness, nausea, and increased pain. He was then transferred to the Surgical Service where, after receiving an additional transfusion, an exploratory laparotomy was done.

At operation, on opening the peritoneal cavity, blood was immediately visible and 5,000 cc. of frankly bloody fluid were removed by suction. The abdominal wall was inspected for bleeding points but none was seen. Then the spleen, mesentery, large bowel, small bowel, stomach, liver, and lesser sac were inspected but no bleeding point was discovered. Following the operation he received additional whole blood transfusions, and had no further bleeding. The patient made an uneventful recovery from the laparotomy.

COMMENT

Although serious complications of pneumoperitoneum are not common, the case reported above is an example of a complication which might have been fatal without therapy. Although the source of hemorrhage was not discovered, it seems most likely that it either resulted from puncture of a vessel in the abdominal wall just beneath the parietal peritoneum of the abdominal wall or from a ruptured vascular adhesion, particularly in view of the tearing sensation in the abdomen just before the onset of the pain. Hemorrhage into the pleural cavity has been reported during treatment with pneumothorax by Holden (4) who believed that tearing of a vascular adhesion was the cause of bleeding. Thus the events in this case of pneumoperitoneum could have been similar to a complication known to occur in pneumothorax. There was no additional bleeding following the laparotomy, possibly due to a spontaneous cessation of hemorrhage or perhaps as a result of the intraperitoneal deflation with relaxation of an injured blood vessel previously held taut by the positive pressure of the pneumoperitoneum.

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NOTES

A TUBERCULOSTATIC AGENT PRESENT IN ANIMAL TISSUES

In the course of a program of studies aimed at analyzing the nonimmunological factors that affect the course of tuberculous infection, we have recognized the presence in a variety of animal tissues of a substance which exerts a pronounced bacteriostatic effect on tubercle bacilli.

The substance can be prepared as follows: The kidneys from guinea pigs freshly killed are broken up in a Waring Blendor. The tissue is washed at neutrality with 50 per cent ethanol, and the washings are discarded. The solid residue is then extracted at pH 4.5 with 50 per cent ethanol. The active material is present in the fraction soluble in acid alcohol and can be separated from it by precipitation with acetone. It can be desiccated by washing with acetone-ether mixtures. The active fraction present in the desiccated material can again be dissolved in water at neutrality.

The antibacterial activity of the material can be tested by adding dilutions of it to liquid culture media containing 0.5 per cent of oleic acid-albumin complex or 10 per cent of serum. The results obtained are independent of the presence or absence of Tween in the medium. Complete inhibition of growth of tubercle bacilli can be obtained even when large inocula are used, but the material does not exert any detectable bactericidal effect.

The purification of the tuberculostatic agent has not yet been carried far enough to allow a statement of its biological activity or of its nature. Suffice it to say that, in some preliminary experiments, inhibition of growth could readily be obtained with final dilutions of the order of 1:100,000 in albumin or serum media. The inhibitor has now been separated from several organs of cattle, rabbits, and guinea pigs. A substance with similar properties is also present in egg white, from which it can be prepared by a method similar to the one used for the extraction from animal tissues. The tuberculostatic agent is present in preparations of lysozyme obtained from egg white, but it appears to be different from lysozyme. The inhibitor is inactivated to a large extent by agar and is, therefore, best tested in liquid media.

Strains of tubercle bacilli differ somewhat in their susceptibility to the bacteriostatic effect. Of particular interest is the fact that BCG cultures as well as cultures of other attenuated strains, such as RuRv, are much more readily inhibited than are the fully virulent strains so far tested.

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CLINICAL STUDIES, TUBERCULOSIS

Ballooned Cavities.—The diagnosis of tension cavity should only be made when there is a rapid increase in size associated with persistent positive intracavitary pressures. These positive intracavitary pressures, however, are rarely found. In most patients who roentgenographically appear to have tension cavities, intracavitary readings reveal pressures oscillating around zero. This is true even for cavities which show demonstrable ballooning a few hours before readings are taken. Other cavities show alternating positive and negative pressures on different days without change in size. The classical theories do not satisfactorily explain how tuberculous endobronchitis and narrowing of the draining bronchus create a ball valve effect. One cannot attribute this to the physiological diminution of the bronchial caliber during expiration. While this is undeniably present in healthy bronchi, it seems unlikely in a diseased segmental bronchus, the infiltrated wall of which has lost its mobility. If a ball-valve mechanism existed, opening of the bronchus would occur on inspiration when the intracavitary pressure was lower than the intrabronchial pressure. At the end of inspiration, the intrabronchial pressure is, at most, equal to zero. If the valve worked ideally, the cavity would equilibrate its pressure with that of the bronchus. In reality, distortion and narrowing of the bronchus will lead to a drop in bronchial pressure. Thus, this mechanism could not explain highly positive intracavitary pressure. The following alternate hypothesis is proposed: There is narrowing of the draining bronchus due to tuberculous endobronchitis which hinders the passage of air in both directions. If the lung containing the cavity cannot produce a respiratory effort equal to that of the contralateral side, a reflux of air ("inverted ventilation") takes place during normal respiration and, in particular, during cough. Bronchial pressures during cough can reach 60, 80, and even 100 cm. of water. It is this reflux of air with its momentary increase in pressure which distends the cavity. The increase of volume may persist after pressure conditions have returned to normal, possibly due to inflammatory changes in the wall and the pericavitary tissue.—*Essai sur la physiopathologie des distensions cavitaires, M. H. Gastaldy, Rev. de la tuberc., 1950, 14: 276.*—(*V. Leites*)

Bronchiectasis and Tuberculosis.—The pathogenesis of bronchiectasis follows a pattern of bronchial obstruction, infection, and atelectasis. An acute respiratory infection may give rise to mucous secretions which can obstruct a bronchus; atelectasis and, eventually, bronchiectasis may result. The factors necessary for the development of bronchiectasis occur in the course of primary pulmonary tuberculosis. Bronchial obstruction may be due to the extrinsic pressure of enlarged lymph nodes or to intrinsic tuberculomata, granulation tissue, ulceration, or calcareous deposits. Atelectasis not uncommonly follows. Eight children were found to have asymptomatic bronchiectasis following primary tuberculosis. The site of the bronchiectasis in each was identified with the original tuberculous lesion. In 6 instances the primary tuberculosis was characterized by definite atelectasis. The duration of the obstruction resulting in bronchiectasis was one to three years. It is believed that the obstructive lesion must be present six months or more before bronchiectatic changes occur. The role of atelectasis as a precursor to bronchiectasis makes further study of this common sequel to primary tuberculosis mandatory. Bronchoscopy, bronchography, or

tomography may demonstrate obstructive bronchial lesions. In the presence of intrinsic or extrinsic obstructive lesions, repeated daily steam inhalations, postural drainage, and breathing exercises should be employed to liquefy tenacious secretions and enhance their discharge. The removal of secretions may transform complete into partial occlusion, relieve atelectasis, and prevent bronchiectasis. The value of streptomycin in the treatment of tuberculous adenopathy is still in dispute but a trial may be warranted since no more specific form of therapy is available.—*Asymptomatic bronchiectasis, a sequel to primary pulmonary tuberculosis, E. Levitsky, P. Cohen, & F. C. Levin, Quart. Bull., Sea View Hosp., April, 1949, 11: 62.*—(*K. T. Bird*)

Spleen and Liver Tuberculosis.—Calcifications in the spleen, in the liver, and in the upper part of the abdomen were noted in 13 children with hematogenous tuberculosis. The calcifications in the spleen were situated mostly in its lateral segment; those in the liver were seen in its lower part near the gall bladder. It is likely that calcifications situated in other segments of the liver are not seen because of the thickness of the parenchyma. The enlargement of the spleen was not in direct relation with the number and size of the calcified tuberculous nodules. There were more girls than boys in this series. The size of the calcific nodules was 1 to 3 mm. in diameter; one calcification of 7 mm. in diameter noted in the spleen was probably due to a lymph node in the hilum. In 4 cases the calcifications in the spleen were not accompanied by calcifications in the liver. These findings were mostly associated with other tuberculous lesions such as spondylitis, active pulmonary tuberculosis, renal tuberculosis, and, in 4 cases, with generalized lymph node tuberculosis.—*Tuberkulose Milz-und Leberherde im Röntgenbild, R. W. Muller, Fortschr. a. d. Geb. d. Rontgenstrahlen, July, 1949, 71: 459.*—(*L. Molnar*)

Immobilization for Pulmonary Tuberculosis.—Remarkable clearing of pulmonary lesions was observed in 40 patients who had prolonged, complete immobilization in plaster casts for associated skeletal tuberculosis. This suggests that certain forms of pulmonary tuberculosis may be treated by immobilization in a plaster cast reaching from shoulders to hips, but not limiting the movements of extremities. This treatment is presently being studied in the French Student Sanatorium.—*Traitement de la tuberculose pulmonaire pour l'immobilization platrée, R. Cohen, Rev. de la tuberc., 1950, 14: 508.*—(*V. Leites*)

Toxicity in Vitamin D2 Treatment.—Skin tuberculosis was treated in young and robust individuals with a vitamin D2 preparation, vigantol forte, 15 mg. twice a week for three to four months, then, 15 mg. once a week up to a total dosage of 780 to 1,300 mg. over a period of twelve to fifteen months. In children and debilitated persons correspondingly smaller doses were given. The drug was in general well tolerated; in most of the patients the general condition improved considerably. Toxic phenomena were observed in some cases but they were, with few exceptions, benign in character and disappeared after a short interruption of the treatment. The toxic phenomena noted in many individuals were: headache, dizziness, lassitude, pains in the limbs, and various cutaneous manifestations. In some patients abdominal pains with vomiting simulated an acute abdominal syndrome. Albuminuria and hematuria were noted in 6 per cent of the patients. The urine contained increased amounts of calcium carbonate, calcium phosphate, and calcium oxalate. Renal function tests showed delayed elimination of fluids and failure of the kidney to concentrate the urine to a specific gravity above 1.025. In 20 per cent of the patients the blood pressure was increased by 25 mm. of mercury. In some of the patients the blood calcium level rose to 11 or 12 mg. per 100 cc. of serum; in a few cases it reached 13 mg. per 100 cc. In view of the frequency of these toxic changes, it is important to carry out this treatment in institutions or at least under close medical supervision. Weekly urine examination is necessary;

after 4 to 6 doses of vigantol, 15 mg., renal function must be tested, calcium determination made, and the blood pressure measured. When toxic reactions occur, the dosage must be lowered or the treatment interrupted. During the treatment an exacerbation of pulmonary and skeletal lesions was noted in 5 cases; in other cases there was an exacerbation of the skin lesions at the beginning. It is, therefore, necessary to perform periodic radiologic examinations of the chest and to determine the erythrocyte sedimentation rate and the blood count.—*Ueber den Verträglichkeit und die Gefahren der Behandlung der Haut-tuberkulose mit hohen Dosen Vitamin D2, F. Zeller, Deutsche med. Wchnschr., February 17, 1950, No. 7: 237.*—(*L. Molnar*)

Resection for Tuberculosis.—Excisional surgery in pulmonary tuberculosis is a selective procedure to be used where collapse therapy is likely to fail or has been a failure. The eradication of all active uncontrolled tuberculous foci is the guiding principle in selecting cases for segmental pulmonary resection, lobectomy, or pneumonectomy. In practice, cases are chosen in which: (*1*) the patient would not or did not benefit from less drastic procedures; (*2*) there are no contraindications to the removal of diseased lung tissue; and (*3*) contralateral lesions are either inactive or under control. Routinely, patients are given streptomycin, 0.5 gm., twice daily intramuscularly for two weeks before operation. Starting two days before operation, the patient receives vitamin C, 200 mg., twice daily and penicillin, 50,000 units, intramuscularly every four hours. Operation is performed in the face-down, slight Trendelenberg position. A closed system of intratracheal anesthesia is employed and the intratracheal tube is left in place until the patient has reacted after the operation. Thereafter, deep breathing and coughing are encouraged; transnasal tracheal suction is used if necessary.

Drainage is used after pneumonectomy only if there has been gross contamination of the pleural cavity or if the parietal pleura has been removed because of empyema. Drainage, usually for forty-eight hours, is performed routinely after partial resection; this removes intrapleural exudate and allows better re-expansion of the remaining lung tissue. Early postoperative ambulation is practiced. Only one tuberculous spread occurred in 54 patients after pulmonary resections, probably due to the prophylactic use of streptomycin, careful anesthesia, and early ambulation. In 10 instances collapse therapy had not been attempted; ipsilateral pneumothorax was induced in 40 patients and contralateral pneumothorax in 7. Four of these patients had contralateral pneumothorax at the time of resection. In this series, there were 47 pneumonectomies, 6 lobectomies, and one lobectomy with resection of the dorsal segment of the lower lobe. In 18 patients (33 per cent) the postoperative course was complicated; 7 (13 per cent) died, all after pneumonectomy. Five of these patients died of causes directly related to the operation, while the remaining 2 died of causes indirectly related. Of the surviving 47 patients, 45 (96 per cent) had no tubercle bacilli in the sputum postoperatively. —*Pulmonary resection for caseous pneumonic tuberculosis, L. A. Hochberg, L. R. Davidson, & I. Fink, Quart. Bull., Sea View Hosp., April, 1949, 11: 45.*—(*K. T. Bird*)

Pulmonary Resection for Tuberculosis.—The indications for pneumonectomy and lobectomy are discussed on the basis of personal experience. Bronchial tuberculosis which leads to stenosis and atelectasis constitutes a definite indication for pneumonectomy; if the stenosis occurs in a lobar bronchus, a lobectomy may be sufficient. Irreversible changes due to caseous pneumonia in a lobe or in a whole lung also indicate the resection of the diseased part. Relative indications for pulmonary resection are (*1*) cavities which do not respond to collapse therapy, particulary those in the apex of the lower lobe, and (*2*) residual cavities after thoracoplasty. Tuberculomas may also be removed by pulmonary resection. When a less extensive operation may control the disease, pulmonary resection should not be done. The removal of a whole

lung must usually be followed by an extensive thoracoplasty. Five operative deaths were observed in 28 consecutive cases of pneumonectomy. One patient, a 58-year-old woman, died of heart failure; one died of hemorrage; 2, from spread to the contralateral lung; and 1, from tuberculous empyema caused by bronchial leakage. Among the 28 cases of pneumonectomy, there were 4 patients with a leaking bronchus, 3 of which healed with Buelau drainage and thoracoplasty; one died six months later from tuberculous pericarditis. Six cases of lobectomy were performed for bronchial tuberculosis, all of them with good results.—*Die Lungenresektion bei der chirurgischen Behandlung der Lungentuberkulos, V. A. Brunner, Deutsche med. Wchnschr., April 14, 1950, No. 15: 501.*—(*L. Molnar*)

Nervous Disorder after Phrenic Crush.—A syndrome closely resembling that of posttraumatic lesions of reflex origin in the vegetative nervous system was observed after an operation on the right phrenic nerve. A few hours after the intervention, intense pain in the right shoulder began and radiated toward the lower part of the ipsilateral hemithorax; paresthesias appeared in the arm and hand on the same side. The pain, continuous in character with exacerbations, was characterized by an intense burning sensation which lasted for approximately fifteen days. Transitory paresis of the right hand was noted. Three days after the intervention, dark pigmented areas appeared on the dorsal surface of the right thumb and index, less on the small finger. Approximately one month later the whole symptomatology disappeared.—*Sindrome simpatico ocasionado por una frenicoexeresis, A. Bosch Millares, Rev. espan. de tuberc., March, 1950, No. 180: 207.*—(*L. Molnar*)

CLINICAL STUDIES, NONTUBERCULOUS DISEASES

Pleurodynia and Coxsackie Virus.—During the course of studies to determine whether there was evidence of interference between the Coxsackie virus and mouse-adapted strains of poliomyelitis, it was discovered that intranasally Coxsackie virus 2 could infect the human adult, producing symptoms of fever, malaise, and pleuritic-type chest pain resembling symptoms of epidemic pleurodynia after an incubation period of forty-six hours. The virus was isolated from the nasal washings and blood of an adult with the disease. It was found that complement-fixing and virus-neutralizing antibodies develop within three days after the onset of symptoms. Twenty patients involved in 3 small epidemics of pleurodynia showed complement-fixing and virus-neutralizing antibodies against Coxsackie virus 2 but showed no reaction to Coxsackie virus 1 or 3 or with normal mouse antigen. Negative complement-fixation tests were demonstrated in 26 healthy adults with no history of symptoms suggesting Bornholm disease. Sporadic cases of pleurodynia variously diagnosed as coronary thrombosis, nonparalytic poliomyelitis and intercostal neuralgia have shown antibodies against the Coxsackie virus. The evidence suggests that the Coxsackie virus is widely distributed in Great Britain and that some epidemics of pleurodynia are associated with infection by Coxsackie virus 1 or 2 or very closely allied viruses.—*Coxsackie viruses and Bornholm disease, G. M. Findley & E. M. Howard, Brit. M. J., May 27, 1950, No. 4664: 1233.*—(*E. A. Riley*)

Bornholm Disease.—Eleven cases of benign dry pleurisy (epidemic pleurodynia, Bornholm's disease, epidemic myalgia) occurred in two adjacent lodging houses in London during September and October, 1949. The patients were young adults between the ages of 19 and 28 years. The onset was sudden with the presenting symptom of pleuritic pain, usually worse on lying down on the affected side. The position and reference of the pain suggested a diaphragmatic origin. The average duration of illness was three to four days and there were no complications. A pleural friction rub was heard in 2 patients. The incubation period varied from two to nineteen days, the cases occurring in 2 groups at intervals of ten days.

The first cases in each group were a cook and foodhandler, respectively, suggesting an alimentary spread at the onset. Sera from 5 patients were tested with the complement-fixation test for Coxsackie 2 virus; all were positive and 2 normal controls were negative. Concomitantly with the epidemic, one sporadic case was seen in which the complement-fixation test for the Coxsackie 2 virus was also positive.—*Epidemic of benign dry pleurisy, D. G. H. Sylvester, Brit. M. J., September 16, 1950, No. 4680: 653.*—(*E. A. Riley*)

Pulmonary Involvement in Typhoid.—Evidence of pulmonary involvement was found in 64 per cent of 80 cases of typhoid and paratyphoid fevers occurring in Eygptian males. In 42 per cent these signs were moderate or prominent in degree. Rales, rhonchi, decreased breath sounds, squeaks, dullness, and wheezes occurred in that order of frequency. They were also found in combination with each other. Frank pneumonia was found in 11 per cent of the patients, but in none was the typhoid or paratyphoid organism the primary cause. A primary pneumonia in which the etiologic agent is one of the enteric group of *Salmonella* organisms is comparatively rare. Bacteriologic studies of the bronchial secretions and sputum in 19 patients failed to reveal any constant pathogens which could be related etiologically to the pulmonary signs. Positive physical signs in the group studied were not confined to the most severely ill patients.—*Pulmonary involvement in typhoid and paratyphoid fevers, F. A. Nesa, Ann. Int. Med., July, 1950, 33: 83.*—(*T. H. Noehren*)

Pulmonary Effects of Gasoline.—Pulmonary involvement becomes evident within a few hours after the aspiration of gasoline. Cases have been reported in which simple pneumonitis, sterile purulent effusion, bloody effusion, and pulmonary abscesses were noted. Lesions occur predominantly in the lower lobe of the right lung and are accompanied by leukocytosis and fever. Dizziness, headache, coma, hilarity, neuritis, and other evidence of nervous system involvement have been observed. A case is presented in which these manifestations followed an attempt to siphon gasoline from an automobile tank. While the reports of gasoline intoxication following aspiration are rare, in each one the pulmonary disease followed accidental aspiration of the liquid material that was being siphoned from a container. In each case respiratory symptoms developed within a few hours after the accident.—*Pulmonary manifestations of gasoline intoxication, R. Zucker, E. D. Kilbourne, & J. B. Evans, Arch. Indust. Hyg. & Occup. Med., July, 1950, 2: 17.*—(*T. H. Noehren*)

Pneumonia in Children.—A total of 91 cases of pneumonia in children were treated by a single injection of procaine penicillin G suspended in peanut oil containing 2 per cent aluminum monostearate. The dosage varied according to the weight of the patient from 300,000 units for those weighing up to 10 lbs. to 1,200,000 units for those weighing 30 lbs. and over. High blood levels of penicillin were found in the first hour following the injection. Accepted therapeutic levels for pneumococci were universally present for four days. Good results were obtained in 96 per cent of the cases.—*Treatment of pneumonia in children with a single injection of repository penicillin: A study of height and duration of penicillin levels, J. F. Crigler, J. N. Lysaught, & E. G. Fisher, J. A. M. A., June 10, 1950, 143: 532.*—(*H. Abeles*)

Pleural Fluid Eosinophilia.—The literature was reviewed and a few reported cases of pleural fluid eosinophilia were found. Only those in which the eosinophilia was greater than 5 per cent were included. A great variety of unrelated pulmonary and pleural disease such as pneumonia, tuberculosis, neoplasms, allergic disorders, and others were found. No common denominator existed. Usually there was a blood eosinophilia, but not always. The authors report a case each in the course of lobar pneumonia, an indefinite allergic disorder, and Hodgkins' disease.—*Pleural fluid eosinophilia, F. A. Mac Murray, S. Katz, & H. J. Zimmerman, New England J. Med., August 31, 1950, 243: 330.*—(*A. G. Cohen*)

Pleural Effusion in Cirrhosis.—In a patient with Laennec's cirrhosis of the liver and ascites, a peritoneopleural fistula became apparent following an episode of fever and cough. Evidence for communication was obtained by the rapid passage of dye from the ascites into the pleural effusion and by the induction of a hydropneumothorax by the intra-abdominal instillation of oxygen. It is suggested that, while pleural effusion is an uncommon complication of cirrhosis, it warrants a similar type of study in other patients with this condition (Author's summary.)—*Pleural effusion produced by abdomino-pleural communication in a patient with Laennec's cirrhosis of the liver and ascites, M. H. Williams, Jr., Ann. Int. Med., July, 1950, 33: 216.*—(*T. H. Noehren*)

Meig's Syndrome.—A case is reported of ascites and right hydrothorax. Within a year, the patient had developed a bilateral hydrothorax, went downhill, and died. At autopsy, a papillary adenocarcinoma of the left ovary was found. However, both gross and microscopic examination of the lungs, pleura, and peritoneum failed to reveal metastases.—*Meigs' syndrome, O. D. Beresford & R. Aidin, Lancet, August 5, 1950, 2: 211.*—(*A. G. Cohen*)

Smoking and Bronchiogenic Carcinoma.—The incidence of bronchiogenic carcinoma has greatly increased in the last half-century. A study was undertaken to determine by clinical investigations, statistical methods, and experimental studies, the importance of various exogenous factors that might play a role in the induction of bronchiogenic carcinoma. Special attention was paid to the relative importance of previous diseases of the lungs, rural and urban distribution of patients, various occupations and hereditary background, as well as smoking habits. The smoking habits of patients were classified in 5 groups from nonsmokers to chain smokers. A total of 684 patients from various parts of the country with proved bronchiogenic carcinoma were investigated by personal interviews or by questionnaires. The group consisted of 605 male and 25 female patients with epidermoid, undifferentiated, and unclassified carcinomas and 39 male and 15 female patients with adenocarcinomas. Of the 605 male patients with bronchiogenic carcinomas other than adenocarcinoma, 79.3 per cent were 50 years of age or older. There were 51.2 per cent excessive and chain smokers among these 605 patients as compared to 19.1 per cent in the general hospital group. The difference is statistically significant. The occurrence of carcinoma of the lung in a male nonsmoker or minimal smoker is a rare phenomenon (in 2 per cent of the 605 cases). Of the patients with carcinoma of the lungs who had a history of smoking, 96.1 per cent had smoked for over twenty years. There may be a lag period of ten years or more between the cessation of smoking tobacco and the occurrence of the clinical symptoms of cancer. Since few women have smoked for such a long time, this may be one of the reasons for the greater incidence of the disease among men today. The prevalence of cigarette smokers as compared to pipe and cigar smokers is greater among the cancer patients than among the general hospital population of the same age group. The greater practice of inhalation among cigarette smokers is believed to be a factor in the increased incidence of the disease. The influence of tobacco on the development of adenocarcinoma seems much less than on the other types of bronchiogenic carcinoma. The temptation is strong to incriminate excessive smoking, and in particular cigarette smoking, over a long period as at least one important factor in the striking increase of bronchiogenic carcinoma.—*Tobacco smoking as a possible factor in bronchiogenic carcinoma: A study of six hundred and eighty-four proved cases, E. L. Wynder & E. A. Graham, J. A. M. A., May 27, 1950, 143: 329.*—(*H. Abeles*)

Cancer and Tobacco Smoking.—Since 1938 a history of tobacco usage has been obtained routinely as part of the regular clinical history from all patients admitted to the Roswell Park Memorial Institute in Buffalo. The

histories record the date smoking began, duration, type of smoking, and amount per day. This report is based on a study of 1,045 male cancer patients and 605 male noncancer patients. The cancer sites selected were lung, lip, pharynx, esophagus, colon, rectum, and a scattered number of other sites. The noncancer patients were those with symptoms referable to the same sites but of proved noncancerous etiology. Cancer of the lung occurred more than twice as frequently among those who had smoked cigarettes for twenty-five years than among other smokers or nonsmokers of comparable age. Pipe smokers and cigar smokers had no more cancer of the lung than did nonsmokers. Lip cancer was significantly increased among pipe smokers but not among cigarette smokers. No other site of cancer that was included in the study was found to be associated with any particular type of smoking.—*Cancer and tobacco smoking: A preliminary report, M. L. Levin, H. Goldstein, & P. R. Gerhardt, J. A. M. A., May 27, 1950, 143: 336.—(H. Abeles)*

Bronchogenic Carcinoma.—Of 91,295 admissions to Fitzsimons General Hospital from January 1944 to December 1948, 91 patients had carcinoma, proved by histologic examination. Eight per cent of these were between the ages of 22 and 30. In 22 the tumor was removed by pneumonectomy, in 6 by lobectomy, and in one by partial lobectomy. There were 3 operative deaths. Four of the patients in this series were alive two and one-half years or more following surgery. In about 50 per cent resections were considered palliative. Palliative resections are considered justifiable because the risk is not excessive, the patient's remaining span of life is more comfortable, and the average survival period is increased. Radiation therapy is of doubtful value. The apparent or actual increase of incidence of the disease is revealed by the fact that of the deaths in this hospital from 1921 to 1940 cancer of the lung was found at autopsy in 27 as compared to 55 in the period from January 1944 through December 1948.—*Primary bronchogenic carcinoma, J. H. Forsee, J. M. Salyer, & R. T. Gants, U. S. Armed Forces M. J., July, 1950, 1: 738.—(T. H. Nochren)*

Bronchogenic Carcinoma.—Bronchogenic carcinoma is at present the commonest malignant tumor seen at necropsy in the Veterans Hospital at Van Nuys, California. From April 1946 to September 1949, 85 cases were proved by bronchial biopsy or necropsy. Of these, 60 cases were studied at necropsy and are the subject of this study. This series constituted 30 per cent of the malignant tumors seen at necropsy. The hospital population of 1,400 patients from which these cases were drawn was 97 per cent male; 65 per cent of the men were between the ages of 20 and 40, and 32 per cent were over the age of 50. All the chest tumors were in men from 24 to 78 years old, the majority being in the sixth decade. Difficulty often was encountered in determining the anatomic site of origin of bronchogenic carcinoma. For this reason the tumors were classified only as hilar, extra-hilar, and diffuse. In the hilar type the tumors began in a primary or secondary bronchus. In the extra-hilar type the tumor arose distal to a secondary bronchus. In some cases the growth was so diffuse that it was impossible to determine which of these types was present. The hilar type was encountered in 39 cases, the extra-hilar in 19, and the diffuse in 2. There were 40 squamous carcinomas, 18 undifferentiated carcinomas, and 2 adenocarcinomas. The histologic pattern of the undifferentiated pleomorphic carcinomas tended to mimic other malignant neoplasms, particularly those of endocrine glands. In all types widespread metastasis was generally present; there were no metastases in only 4 patients. In 15 patients, mostly of the squamous cell type, the metastases were limited to the thorax. Of interest was the finding of splenic metastases in 6 patients; 3 were oat-cell in type, 2 squamous cell, and one adenocarcinoma. Pulmonary complications were frequent, the most important being atelectasis; less frequently there was suppurative pneumonitis, lipoid pneumonia, chronic bronchiectasis, interstitial myocarditis, active tuberculosis, and silicosis

in descending order of frequency. There was no evidence that tuberculosis or silicosis predisposed to, or protected against, bronchogenic carcinoma. The ultimate prognosis in this series was not dependent upon the cell type, since all patients on whom a histologic diagnosis was made before death expired within a year regardless of therapy. An improved outlook can be attained only through earlier histologic diagnosis and earlier surgical intervention, regardless of cell type of the tumor.—*Bronchogenic carcinoma: A study of 60 necropsies, I. M. Reingold, R. E. Ottoman, & B. E. Konwaler, Am. J. Clin. Path., June, 1950, 20: 515.*—(*J. S. Woolley*)

Bronchiogenic Carcinoma.—The more frequent recognition of bronchial adenomas during life than at necropsy could be due to a change of the benign adenoma to carcinoma before death. The treatment of adenoma should be relatively radical resection, probably a pneumonectomy. Treatment of bronchiogenic carcinoma may become more effective if early pneumonectomy is used. Cough and blood-streaked sputum in middle-aged patients are most suggestive of carcinoma although tuberculosis and bronchiectasis must be considered. Roentgenography, bronchoscopy, and cytologic examination of the bronchial secretions are helpful in 75 per cent of the cases. The remainder may be examined at exploratory operation. The operative mortality of total pneumonectomy is now 10 per cent; 28 per cent of the author's cases have lived more than five years. The incidence of bronchiogenic carcinoma seems certainly to be increasing, perhaps due to some unrecognized exogenous factors which have arisen during the past forty to fifty years.—*Considerations of bronchiogenic carcinoma, E. A. Graham, Ann. Surg., August, 1950, 132: 176.*—(*W. H. Oatway, Jr.*)

Carcinoma of Lung.—In a series of necropsies, there was an incidence of lung carcinoma of 0.6 per cent. These comprised 2.9 per cent of all malignancies and 3.5 per cent of all carcinomas. These figures are much lower than those given for the United States and Great Britain. An inquiry was made into the possible causes. It was found that cigarette smoking previously had increased at a much slower rate in Iceland than in the other countries. Recently, the rate has increased to comparable levels. Assuming that an average of 25 years is required for the development of carcinoma, more cases are to be expected in Iceland about the years 1960 to 1965.—*Lung cancer in Iceland, N. Dungal, Lancet, August 12, 1950, 2: 245.*—(*A. G. Cohen*)

Pulmonary Adenomatosis.—Thirty of the 36 patients with pulmonary adenomatosis reported in the literature were more than 40 years of age. The most constant symptom was cough, usually productive of large amounts of clear, watery, and foamy sputum. Dyspnea, loss of weight, weakness, cyanosis, and the signs of partial consolidation of pulmonary tissue were common. Chest roentgenograms showed either (*1*) discrete nodules or irregular areas of variable size, or (*2*) large areas of density, either segmental or lobar. Most patients died from the disease or its complications. Aspiration biopsy was of help in establishing the diagnosis. Roentgen therapy was of no value; resection was effective in one patient. Three new cases are added to those already reported.—*Pulmonary adenomatosis; clinical review and report of three cases, A. B. Weir, Jr., Arch. Int. Med., May, 1950, 85: 806.*—(*G. C. Leiner*)

Pregnancy Following Pneumonectomy.—A 37-year-old woman had her right lung removed because of grade I adenocarcinoma. The patient became pregnant two years later. The pregnancy and the delivery were completed uneventfully. The load of pregnancy did not change the ventilatory function to any significant extent. The venous pressure rose during pregnancy and remained elevated post partum.—*Cardiovascular and respiratory studies in pregnancy following pneumonectomy, J. H. Long, M. R. Wester, J. R. Willson, & G. P. Rosemond, J. A. M. A., May 27, 1950, 143: 358.*—(*H. Abeles*)

Lobectomy in Aged Patient.—A 79-year-old woman developed an unproductive cough of one week's duration in January 1946. The cough recurred in August 1948. After several months the cough became productive. The patient raised about 2 ounces of thick, purulent sputum daily, the temperature was elevated, and the general condition deteriorated. The chest film showed consolidation of the anterior segment of the middle lobe. There was no response to penicillin treatment. A sputum specimen was reported positive for malignant cells. Bronchoscopy was negative. In February 1949, at the age of 82, the patient was explored and the middle lobe and an adherent segment of the right upper lobe were removed. The pathologic diagnosis was chronic inflammation and bronchiectasis of the middle lobe. There was no evidence of malignant growth. The postoperative course was uneventful except for the development of transient auricular fibrillation. At a follow-up examination eight months after the operation, the patient was in excellent condition and without pulmonary symptoms.—*Lobectomy in a patient eighty-two years of age, M. B. Rosenblatt, A. Aufses, & J. R. Liva, J.A.M.A., June 10, 1950, 143: 552.*—(*H. Abeles*)

Roentgenotherapy in Bronchial Carcinoma.—Since the life expectancy of untreated cases of bronchial carcinoma is not known, the duration of survival after roentgenotherapy is not a reliable criterion of the effectiveness of the treatment. In 78 cases treated with varying dosage schedules, the duration of survival and the treatment schedule showed no statistically significant relation. The radiologic appearance of the tumor under treatment is not a reliable criterion for the effect of roentgenotherapy because a clearing noted immediately after the beginning is usually due to the resolution of inflammatory areas around the tumor; later in the course of treatment, bronchopneumonic patches may appear as a result of the irradiation. Likewise, tomographic examination and bronchography may show post-irradiation changes which cannot be differentiated from shadows caused by the tumor. The only acceptable criterion is clinical observation. In a group of 78 cases, there was a definite improvement of the symptoms in 55 per cent shortly after the beginning of the treatment. The average duration of the improvement was a little less than five months. In 20 per cent of the cases no changes occurred and in 25 per cent there was worsening of the patient's condition so that the treatment had to be interrupted. The average duration of survival was a little more than six and one-half months. It appears, therefore, that roentgenotherapy may give symptomatic relief in bronchial carcinoma. One limitation of such treatment is that a dosage, which affects the tumor, damages components of the pulmonary stroma which would have an important role in the process of replacing the tumor by scar tissue. This damage is particularly evident in subjects in poor general condition. Attempts are being made at present to improve the technique of roentgenotherapy or to use new types of therapy with ultra-penetrating, rapid electronic rays or artificial radioactive substances.—*Ueber den Wert der Röntgentiefenbestrahlung des Bronchialkarzinoms als palliative Massnahme. V. J. Trautmann, Deutsche med. Wchnschr., September 23, 1950, No. 25: 864.*—(*L. Molnar*)

Bony Metastases in Thorax.—Homogeneous densities in the apical and upper lung fields, caused by metastases in the ribs, were observed in 3 cases. At first, such densities appeared to be of pulmonary origin but closer study revealed in all cases destruction of the bony structure of the ribs. The diminished transparency of the lung may be explained by the fact that the tumor mass situated in the rib compressed and displaced the underlying lung. The sharp delimitation of the shadows may be due to the fact that the periosteum sets a limit to the expansion of the tumor. The metastases in the first case were from a primary thyroid adenocarcinoma; in the second and third cases, the source of the metastases was not found. In one case a Pancoast tumor was suspected because of pain in the arm. Metastases in the ribs may lead to

complete decalcification with complete disappearance of the bony structure, isolated erosion, or thinning of the rib due to pressure atrophy and spontaneous fracture. In one case the process was localized to the posterior portion of the ribs; in 2 cases the metastases were situated in the anterior portions.—*Knochenmetastasen im oberen Thorazbereich, G. H. Schmitt, Fortschr. a. d. Geb. d. Rontgenstrahlen, July, 1949, 71: 411.—(L. Molnar)*

Bronchoscopic Cinematography of Bronchitis.—Lipiodal bronchography has made possible more accurate definition and classification of different types of bronchitis. Inflammation of the bronchial tree may be observed through the bronchoscope. Changes in color, secretion, and contour may be noted directly and, with the help of lipiodal filling, can be accurately located in roentgenograms. The bronchoscopic field may be cinematographed in natural colors for later study. With the aid of such procedures, a new classification of bronchitis has been formulated: (*1*) Simple localized bronchitis affecting a main bronchus or its branches. Localization in the branches is indicated by redness, edema, and excessive mucoid or mucopurulent secretion about the orifices in the bronchi. Simple localized bronchitis may be subdivided into: (*a*) seasonal or autonomic bronchitis, and (*b*) recurrent bronchitis, sometimes called chronic bronchitis. (*2*) Associated bronchitis in: (*a*) abscess of the lung, and (*b*) bronchial dilatation. (*3*) Bronchitis associated with cancer of the lung. The etiologic factor in simple localized bronchitis usually moves downward from the upper respiratory tract, including the sinuses in some cases. Sinusitis is, however, less frequently a source of bronchitis than is ordinarily supposed, being a factor in only 20 or 30 per cent of cases. More often, simple chilling or the inhalation of mineral or vegetable dusts brings on the attack. In some cases there is no upper respiratory involvement. Bronchoscopy is rarely required but is indicated if fever and cough persist. Usually more than one lobe or segment is involved. The actual etiologic agent may be any one of various bacteria or viruses, including the influenza bacillus, the tubercle bacillus, and the microorganisms causing other forms of specific disease such as the spirochete of syphilis, the viruses, and various fungi. More diffuse bronchitis may be caused by the inhalation of toxic gases, may follow an operation, or may be a manifestation of an allergic condition. Bronchitis may have an endogenous origin, for example, the bronchitis of a patient with heart disease. Characteristic alterations in the bronchi and their orifices are seen very early through the bronchoscope in cancer of the lung. In cancer of the upper lobe, cinematography is of no value; cancer of the middle lobe can be well studied by motion pictures. In cancer of the base of the lung, bronchial involvment is often limited to one or two segments, and presents a characteristic picture. Early bronchoscopy is necessary in all cases where cancer is suspected.—*De la classification des bronchites et des bronchiectasies et du role du cinéma bronchoscopique, A. Soulas, Le Poumon, May-June, 1950, 6: 239.—(A. T. Laird)*

Tracheobronchial Aspiration.—Recognition of the presence of retained tracheobronchial secretions, a frequent complication in many common medical diseases, necessitates the institution of appropriate measures to effect their removal without delay. Treatment by aspiration of the tracheobronchial tree through a nasally introduced urethral catheter can be undertaken safely and effectively by an internist or general practitioner. Contraindications are local disease in the larynx, glottic spasm, and increased intracranial pressure. In 10 cases the immediate lifesaving effect of this procedure was demonstrated.—*Tracheobronchial aspiration with a urethral catheter, method of treatment and prevention of asphyxial hazards in medical diseases and emergencies, L. Cardon, J.A.M.A., April 8, 1950, 142: 1059.—(H. Abeles)*

Anesthesia for Bronchography.—The difficulties in giving general anesthesia for bronchography in children are: (*1*) the danger of

explosion, and (2) obstruction of the air passage by the catheter or secretions. These difficulties are obviated by the use of a special endotracheal tube with a side tube for instilling radiopaque fluid. The end of the endotracheal tube is attached to the anesthesia apparatus. Preoperatively, postural drainage and penicillin inhalations are given to eliminate secretions. The anesthesia is induced with 5 per cent intravenous sodium thiopentone, 0.5 to 1.0 gm., for a child. The larynx is cocainized by direct laryngoscopy. The endotracheal tube is then passed into the trachea and connected with the apparatus. A mixture of equal parts of nitrous oxide and oxygen is passed over trichlorethylene. This mixture is nonexplosive. Under fluoroscopic guidance, the radiopaque fluid is then introduced. In case of obstruction, suction of the air passage can be performed readily.—*General anaesthesia for bronchography in children, G. L. Way & G. C. W. James, Lancet, June 10, 1950, 1: 1073.*—(*A. G. Cohen*)

ANTIMICROBIAL THERAPY

PAS for Tuberculosis.—Extensive experience with *p*-aminosalicylic acid (PAS) in the treatment of human tuberculosis has shown that much larger doses than those heretofore employed are necessary to obtain blood levels of 15 to 20 mg. Adults require 20 to 25 gm. a day in doses of 2 to 2.5 gm. every two hours during the day and twice during the night. The blood level of the drug should be followed in each case. Even with these dosages, only traces of the drug can be found in the spinal fluid. To attain effective concentrations of the drug in the spinal fluid, it is necessary to administer PAS day and night by continuous intravenous drip for a total of 40 gm. of its sodium salt daily. By this means blood levels of 50 mg. and spinal fluid levels of 15 to 25 mg. are achieved. This explains the failures previously reported in the treatment of miliary tuberculosis and tuberculous meningitis with PAS. It is advisable to use both streptomycin and PAS either simultaneously or successively in gravely ill patients. In order to diminish or prevent the frequent gastrointestinal side effects with PAS, tablets and granules are used, either simultaneously or alternately. If necessary, the treatment should be interrupted for one or two days each week. The continuous intravenous route is advised in the severe acute or subacute disease such as miliary dissemination, tuberculous pneumonia, and subacute spreads; in certain types of old cavitation; and in patients with severe gastrointestinal intolerance. With this regimen results have been definitely superior to those obtained with streptomycin alone. Eighteen cases of early meningitis diagnosed eight to fifteen days previously, and with no previous treatment except sporadic streptomycin injections, have received the combined treatment. Of these, 12 patients are well eight to eighteen months later; they have no abnormal clinical signs, and completely normal spinal fluid. Four are still under treatment but in good clinical condition. Two died during the first eight days of treatment without emerging from the coma in which they were admitted. Patients with meningitis treated during a later stage of their disease after a relapse following an initial good response to streptomycin alone, or with chronic meningitis previously treated with streptomycin, do not respond as well to the combined treatment. Even these patients occasionally show good but slow results. Of 16 such patients, 2 appear completely well after ten months and one year, respectively; 6 are still under treatment and in good condition; and 8 have died after a prolonged course. PAS alone is effective in tuberculous meningitis. Before streptomycin was available, patients were treated with PAS alone, with results similar to those obtained with streptomycin. PAS alone has been effective also in treating a number of patients in relapse after streptomycin treatment. Taken orally, PAS diffuses rapidly into the pleural space, reaching concentrations in the pleuritic fluid of 20 to 25 mg. per cc. As much as 50 cc. of a 10 per cent solution may be injected intrapleurally. In two years, 22 cases of tuberculous pleurisy have been treated with PAS. No parenchymal lesions have been noted in any of these cases. PAS was employed intra-

pleurally alone or alternately with streptomycin in 17 patients. A dry pleural space was obtained in 14 cases of tuberculous empyema; 3 patients required surgery. PAS has also been used extensively alone and with streptomycin in the treatment of pulmonary tuberculosis, including primary infections in children and adults. Bacillary resistance to PAS, even in cases treated for several months, has not occurred in more than 5 to 10 per cent, in contrast to 70 to 80 per cent in similar cases treated with streptomycin. PAS is a valuable therapeutic agent on a par with streptomycin; less toxic and of more lasting efficacy in general.—*El tratamiento de la tuberculosis por el ácido paraaminosalicílico, J. Paraf, J. Desbordes, M. Paraf, & P. Zivy, Hoja Tisiol., June, 1950, 10: 135.*—(*F. Perez-Pina*)

Infant Tuberculosis.—Of 54 children with primary tuberculosis between the ages of 5 and 28 months, 30 per cent had been infected during the first year of life. They were observed for periods of one to three years. The mortality was 6.5 per cent (3 cases). All the deaths were due to tuberculous meningitis. Two of these children had received prolonged streptomycin treatment before admission, again proving the inability of streptomycin to prevent this complication. In this series of primary tuberculosis, streptomycin was administered only in 5 cases because of the alarming clinical condition. The gravity of primary tuberculosis in the infant seems to have been continuously decreasing. The virulence of the bacillus being the same, this is attributed to a changed mode of infection. Modern control of tuberculosis makes prolonged repeated contamination a much rarer occurrence nowadays. Streptomycin showed no effect on massive hilar adenopathies, on associated atelectasis, or on erosions of the nodes.—*Sur le pronostic et le traitement actuels de la primo-infection du nourrisson, A. Dufourt, J. Paviot, C. Ollaguier, & R. Touraine, Rev. de la tuberc., 1950, 14: 556.*—(*V. Leites*)

Bone and Joint Tuberculosis.—Seven cases of skeletal tuberculosis were treated with streptomycin; results were uniformly good and there were no recurrences. The total dosage of streptomycin was relatively small, from 9 to 120 gm. being used. The total dosage varied with the acuteness of the disease, the location and size of the lesion, general condition of the patient, and the addition or omission of surgery. Surgery should be performed whenever possible and streptomycin should be placed in the operative site. Final evaluation of streptomycin in the treatment of tuberculosis of bones and joints can be made only after prolonged study and observation.—*The treatment of bone and joint tuberculosis with surgery and streptomycin, C. W. Phillips, M. N. Proffitt, & N. C. Jefferson, J. Nat. M. A., July, 1950, 42: 199.*—(*L. Hyde*)

Dihydrostreptomycin Contraindications.—The substitution of dihydrostreptomycin for streptomycin in the treatment of tuberculous meningitis over a period of about one year showed that the former substance is markedly more toxic than the latter. At the same dosages it was found that in adults, but not in children, dihydrostreptomycin caused partial-to-total deafness in a very high percentage of the patients. Since January 1950, 80 per cent of the patients suffered impairment of hearing. Patients requiring antibiotic treatment for more than two months should receive streptomycin, not dihydrostreptomycin.—*Dihydrostreptomycine Tegenaangewezen Bij "Lange" Kuren, J. E. Minkenhof, Nederl. Tijdschr. v. Geneesk., July 22, 1950, 94: 2129.*—(*J. van der Vate*)

Speed of Streptomycin Action in Tuberculosis.—In order to determine the earliest time at which streptomycin begins its action against tubercle bacilli in the guinea pig, 20 animals were inoculated with virulent bacilli and then treated daily with streptomycin. Twenty other similarly inoculated guinea pigs were not treated and served as controls. At five, ten, and fifteen days, 6 animals were sacrificed. Ten centigrams of splenic tissue from each animal were ground and cultured for tubercle bacilli. The total colony count in the streptomycin-treated animals was de-

cidedly smaller than in the control group at five, ten, and fifteen days. These findings do not support the hypothesis that for streptomycin to manifest its effects it is necessary that acquired immunity be present, since the effect of streptomycin was apparent before immunity could have developed. The increase in the number of bacilli during the first few days of streptomycin therapy, however, remains to be explained.—*Momento en que empieza a actuar la estreptomicina en la tuberculosis experimental del cobayo, A. Saenz & G. Canetti, Hoja Tisiol., June, 1950, 10: 148.*—(*F. Pérez-Pina*)

Streptomycin and Tuberculin Allergy.—Tuberculin allergy produced in guinea pigs by the inoculation of living tubercle bacilli is markedly diminished in intensity by the administration of streptomycin. The allergy produced in the guinea pig by the injection of dead tubercle bacilli likewise reveals the depressing effect of streptomycin. It thus seems probable that the desensitizing effect produced by streptomycin in the course of a tuberculous infection is not due only to the sole bacteriostatic effect of the antibiotic (Author's summary).—*Action de la streptomycine sur l'allergie produite par les bacilles tuberculeux vivants et morts, G. Canetti & A. Saenz, Ann. Inst. Pasteur, July, 1950 79: 95.*—(*V. Leites*)

Staining for Streptomycin Resistance.—The usual *in vitro* methods of determining streptomycin resistance demonstrate only the maximum resistance of some of the tubercle bacilli without giving any information on the proportion of bacilli which remain sensitive. A slide method permits the study of bacilli directly without culturing. Smears of sputum or other secretions are placed on slides and treated as follows: (*1*) fix by heating; (*2*) heat for ten minutes in an equal mixture of carbolfuchsin and gentian violet; (*3*) wash with water and stain with nitric acid 1:3; (*4*) wash with water and decolorize with alcohol; (*5*) wash with water and cover with Lugol's solution for five minutes; (*6*) wash with water and counterstain with malachite green. By this procedure, the streptomycin-sensitive bacilli appear as nonhomogeneous bodies of a shade similar to that in the ordinary Ziehl stain with chromophile moniliform granulations. The streptomycin-resistant bacilli are homogeneous without granulations and are brown-red. The proportion of resistant to sensitive bacilli can thus be rapidly evaluated. —*Mesure rapide de la streptomycino—resistance des bacilles tuberculeux donnant le pourcentage de germes sensibles et resistants, R. Benda & D. A. Urquia, Presse med., July, 22, 1950, 22: 841.*—(*V. Leites*)

Streptomycin Resistance.—In 2 patients who received 43 and 40 gm. of streptomycin, respectively, the sputum showed the simultaneous presence of very sensitive and highly resistant tubercle bacilli. Colonies of the sensitive strains had the usual typical appearance, whereas the resistant strains formed flat atypical colonies.—*Présence simultance dans les crachats de bacilles de Koch très resistants det très sensibles a la streptomycine, P. Courmont, P. Galy, & G. Deries, Rev. de la tuberc., 1950, 14: 560.*—(*V. Leites*)

Bacteriostatic and Bacteriocidal Effect of Streptomycin.—Streptomycin-resistant bacilli from treated patients or produced *in vitro* do not show any morphologic changes. Sula's reaction, a blackening of potassium tellurite in the presence of living tubercle bacilli, occurs when cultures sensitive to 2 γ per cc. are left in contact with 1,000 γ per cc. for twelve days. After this lapse of time, sensitive strains lose 60 per cent of their viability but their virulence remains intact. Resistant strains retain their viability as well as their virulence. —*Dissociation des pouvoirs "bacteriostatique" et "bactericide" de la streptomycine in vitro sur le bacille de Koch, F. Tison, Ann. Inst. Pasteur, July, 1950, 79: 100.*—(*V. Leites*)

In Vitro Activity of Thiosemicarbazones.—A number of representative compounds of the thiocarbazones have been synthesized and tested to determine the correlation of their structure with their *in vitro* activities. In the

in vitro test, *Mycobacterium tuberculosis* var. *bovis*, strain BCG was used. The test medium was a modified Kirchner medium in which sorbitan monooleate was replaced by Triton A-20 and in which the serum albumin and iron ammonium citrate were omitted. A compound was considered inactive if it did not inhibit at a concentration of 10 γ per ml.; active, if it inhibited at a concentration of 1 to 10 γ per ml.; and highly active, if it inhibited at a concentration of less than 1 γ per ml. The results showed that thiosemicarbazide and its alkyl derivatives were active *in vitro*, but its acyl derivatives were, in general, inactive. Thiosemicarbazones formed from aliphatic carbonyl compounds were inactive. Condensation of aromatic aldehydes or cinnamic aldehydes with thiosemicarbazide gave active derivatives, but condensation with 3- and 4-substituted thiosemicarbazides gave inactive derivatives. No apparent correlation existed between the substituents in aromatic aldehydes and the activity of their corresponding thiosemicarbazones. Thiosemicarbazones derived from aromatic ketones were active *in vitro* although somewhat less active than the thiosemicarbazone from the corresponding aldehyde. Thiosemicarbazones derived from heterocyclic aldehydes were also active although somewhat less active than benzaldehyde thiosemicarbazone (Authors' summary).—*The chemotherapy of experimental tuberculosis: I. The in vitro activity of thiosemicarbazides, thiosemicarbazones, and related compounds, R. Donovick, F. Pansy, G. Stryker, & J. Bernstein, J. Bact., May, 1950, 59: 667.*—(*F. G. Petrik*)

Thiosemicarbazones in Mouse Tuberculosis.—A standardized screening test using mice was employed to evaluate a series of thiosemicarbazones and related compounds. Groups of 10 mice of the CF1 strain, each animal weighing 17 to 19 gm., were inoculated intravenously with the Ravenel strain of *Mycobacterium tuberculosis*. The drug was given in the diet at two or three dosage levels for 21 days beginning 48 hours before inoculation. Each experiment included groups of untreated infected mice as well as groups treated with *p*-aminosalicylic acid at 0.375 per cent and 0.75 per cent in the diet. Nearly 100 thiosemicarbazones and related compounds were tested for antituberculous activity; only 8, all of these being *p*-substituted benzaldehyde thiosemicarbazones, showed significant reproducible activity. In order of activity, the substituents ranked as follows: ethyl sulfonyl and isopropyl were more active than amino, acetamido, and dimethylamino, which were, in turn, more active than nitro, sulfamyl, and methoxy benzaldehyde, 3-thiosemicarbazone. There was no quantitative relationship between *in vivo* and *in vitro* antituberculous activity, even among active compounds within the series of the thiosemicarbazones; however, thus far only compounds active *in vitro* have been found to be active *in vivo*.—*The chemotherapy of experimental tuberculosis: II. Thiosemicarbazones and analogues in experimental tuberculosis in the mouse, D. Hamre, J. Bernstein, & R. Donovick, J. Bact., May, 1950, 59: 675.*—(*F. G. Petrik*)

Antituberculosis Agents in Mice.—Male white mice weighing between 18 and 20 gm. each were infected intravenously with a human mixture of *Mycobacterium tuberculosis*, Strain 905. An acute infection was produced with 0.75 mg. and a "chronic" infection with 0.02 mg. of culture. *p*-Aminosalicylic acid (PAS) was mixed with the food to the extent of 1 per cent, thus ensuring an intake of about 40 mg. of drug per mouse per day. Streptomycin, 1,000 units, was given subcutaneously twice a day. *p*-Ethylsulphonylbenzaldehyde thiosemicarbazone (No. 8388) was given by admixture with the food to the extent of 0.25 per cent, thus ensuring an intake of about 10 mg. of drug per mouse per day. Three sets of experiments were carried out: (*1*) The drugs were given continuously from the day of inoculation until death; (*2*) the drugs were given for the first two weeks after inoculation; (*3*) treatment with streptomycin or thiosemicarbazone was begun on the twenty-first day after inoculation and continued until death. The progress of the infection was assessed by

recording all deaths and noting the presence or absence of specific lesions post mortem. The thiosemicarbazone showed higher activity than PAS and was superior to streptomycin in retarding the early stages of the disease.

In late stages, however, streptomycin was more effective. In the assessment of therapeutic activity, the survival time of treated mice reflected accurately the final state of the disease. Variations in the course of the disease and differences in the actions of the drugs could only be studied by correlating the growth, mortality, and pathologic changes in treated groups of mice. Therapeutic activity in the compounds tested was comparable in effect to reducing the numbers or the virulence of the bacilli inoculated. True remission or sterilization of the infection did not occur.—*The chemotherapy of experimental tuberculosis in mice, A. R. Martin & G. T. Stewart, Brit. J. Exper. Path., 1950, 31: 189.—(H. J. Henderson)*

Benzyl Furamide in Experimental Tuberculosis.—When guinea pigs were treated with N-benzyl-2-furamide following infection with H37Rv tubercle bacilli, they did not develop less tuberculosis than control untreated animals. Those treated at the same time with streptomycin alone or in combination with the galacturonic acid derivative of 4,4'-diaminodiphenylsulfone showed good resistance, and the latter drug alone showed about one-third the activity of streptomycin alone. These results are at variance with those of Shpanir and Chertkova who used N-benzyl-2-furamide in mice.—*Action of N-benzyl-2-furamide in experimental tuberculosis: Comparison with a sulfone and streptomycin, M. I. Smith, H. Bauer, Y. T. Chang, & H. Friedlander, Proc. Soc. Exper. Biol. & Med., April, 1950, 73: 563—(F. B. Seibert)*

LABORATORY STUDIES

M. Tuberculosis in Egg Yolk Media.—Egg yolk can be used as the basis for easily prepared liquid and semi-liquid media which will support the rapid and heavy growth of small inocula of tubercle bacilli. Water-clear extracts of egg yolk can be prepared by removing the acetone soluble fats, and these extracts will also support growth from small inocula.—*Growth of Mycobacterium tuberculosis in egg yolk mediums, A. H. Eggerth, Proc. Soc., Exper. Biol. & Med., April, 1950, 73: 542.—(F. B. Seibert)*

BCG.—Freeze-drying (lyophilization) is the only way to preserve BCG for transportation over long distances and for distribution in tropical countries. The method was first devised in Russia in 1937 but did not become known outside that country until 1947. The best excipient found so far among many tested is 50 per cent glucose. By *in vitro* experiments, it was shown that there was no difference in the number of colonies before and after freeze-drying when the tubes were kept refrigerated. When stored at room temperature (18° to 25°C.), there was a 20 per cent decrease in the number of living bacilli at the end of the first month and a slow decrease over the next five months to 40 per cent. Even after eleven months, colonies could be obtained. When the tubes were incubated at 38°C., there was an important decrease during the first week and then the cell count stabilized. Living bacilli could be obtained but in numbers insufficient to allergize guinea pigs. *In vivo* experiments showed that BCG after freeze-drying was as effective as the original strain in producing allergy in the guinea pig but a little longer time was required. Resistance studies also showed equal protective power before and after lyophylization.—*Freeze-dried (lyophilized) BCG, F. Van Deinse & F. Sénéchal Tubercle, July, 1950, 31: 157.—(A. G. Cohen)*

Intravenous Tuberculin and Leukocytes.—Following the intravenous injection of 0.25 or 0.50 mg. PPD into tuberculous rabbits there is a decrease in the total number of circulating white cells and especially in the lymphocytes. The maximum decrease occurs in six to nine hours and is followed by a gradual return to normal. There is also frequently a delayed increase in neutrophils.—

Effect of intravenous injection of tuberculin on leukocytes of normal and tuberculous rabbits, N. G. Brandt, C. N. Sandage, & J. M. Birkeland, Proc. Soc. Exper. Biol. & Med., June, 1950, 74: 315.—(F. B. Seibert)

Nature of Tuberculin.—Surface growth of tubercle bacilli for two months at 37°C. on the simple, synthetic, nonprotein Wong-Weinzirl medium resulted in a good yield of bacillary mass and the liberation of about 10 mg. of tuberculoprotein per 100 ml. of filtrate. The reaction of the medium in the meantime had changed from pH 7.0 to below pH 6.0. Reinoculating the Seitz filtrate with or without heating resulted in a further growth of bacilli within two months without an appreciable increase in the tuberculoprotein. Heating the filtrate without neutralizing it before reinoculation reduced the amount of tuberculoprotein. Other cultures were kept undisturbed for more than eight years at incubator temperature. This resulted in a definite loss in bacillary weight but there was about as much tuberculoprotein remaining in the filtrate as at three to seven months' incubation. No disintegration of the tuberculoprotein was evident over this long period, nor was the biological activity diminished. It is commonly believed that tuberculin is not heat coaguable, but heating at a pH below 6.0 resulted in a precipitate or coagulum with a loss in the tuberculin potency of the filtrate. This showed that tuberculin is partially denatured by heat when the pH is 6.0 or less. In 1:100 and 1:1000 solutions, properly buffered at pH 7.0, free from preservatives or contamination and in sealed amber-glass ampules, tuberculin is biologically stable for more than nine years at room or refrigerator temperatures, but not at incubator temperature. Dilutions in unbuffered saline solutions are not stable. It appears that the tubercle bacilli possess autolytic enzymes capable of producing tuberculin *in vitro* but not capable of carrying the active material beyond the specific biologically active protein stage.—*The nature of tuberculin, H. J. Corper & M. L. Cohn, Am. J. Clin. Path., July, 1950, 20: 605.—(J. S. Woolley)*

Decompression Injury.—The results of over 600 experiments involving 350 rats are reported. All animals survived explosive decompression to 21 mm. of mercury even when repeated as many as twenty times in as many minutes, when immediately recompressed, in less than one second. Survival was found to be associated with time under reduced pressure rather than with pressure change *per se*. The end results of nitrogen breathing were found to parallel those of explosive decompression in both incidence of pulmonary hemorrhage and lethal time. Young rats weighing 10 to 30 gm. survived nitrogen breathing as well as anoxia following explosive decompression for periods much in excess of those tolerated by adult animals. Aeroemboli were never observed in the jugular, mesenteric, renal, or femoral vessels of rats maintained until death at 21 mm. of mercury following explosive decompression. Injections of the autonomic drugs, atropine and mecholyl, significantly reduced the mortality in rats exposed to a standardized decompression invariably lethal to untreated animals. Anesthesia by means of ether, evipal, or pentothal permitted all rats tested to survive an explosive decompression uniformly fatal to control animals. On the basis of these and earlier experiments, it is suggested that the duration of post-decompression anoxia represents the major factor in determining the outcome of an explosive decompression (Authors' summary).—*Etiology of explosive decompression injury, E. L. Corey & E. G. Lewis, Am. J. Physiol., August 1, 1950, 162: 452.—(G. C. Leiner)*

Respiration during Exercise.—The behavior of pulmonary ventilation, oxygen consumption, arterial pCO_2, and arterial (H+) was studied during electrically-induced exercise in the barbitalized dog. Ventilation was a linear function of oxygen consumption over a range of the latter extending from 60 to 665 cc. per minute. The relationship did not differ significantly from a direct proportion. Tidal volume was also a linear function of oxygen consumption within the range of the present data, whereas respiratory rate increased

hyperbolically. No significant change in arterial pCO_2 occurred during exercise, as shown by the absence of correlation between this variable and metabolic rate. The characteristic change in acid-base balance associated with exercise in these experiments was a poorly compensated metabolic acidosis, the severity of which increased with the metabolic rate. In the animals, as in man, changes in arterial pCO_2 and for (H+) cannot account for the entire ventilatory response to exercise. In both, a ventilatory stimulus of potency directly proportional to the metabolic rate, operating in addition to these chemical agents, is capable of accounting for the observed responses. (Authors' summary).—*Regulation of breathing during electrically-induced muscular work in the intact anesthetized dog, D. P. Morgan & F. S. Grodins, Am. J. Physiol., July 1, 1950, 162: 54.*—(*G. C. Leiner*)

Respiration during Exercise.—The respiratory responses of barbitalized dogs to electrically induced exercise were studied following transection of the spinal cord at T 10, and the results were compared with those previously obtained in the intact animal. A significant positive correlation was found between ventilation and oxygen consumption in the transected animals, but the relationship was curvilinear in contrast to the direct proportion found in the intact dog. A significant positive correlation was found between arterial pCO_2 and oxygen consumption in the transected animals in contrast to the lack of relationship between these variables in the intact animal. A significant positive correlation was found between arterial (H+) and oxygen consumption in the transected animals. This was also true in the intact animal, but the increase in (H+) per unit increase in oxygen consumption was less in the latter. The predominant change in acid-base balance associated with exercise in the transected animals was a respiratory acidosis. This is in contrast to the metabolic acidosis observed in the intact animals. Quantitative analysis revealed that the chemical agents, pCO_2 and (H+) contributed approximately 40 per cent of the total ventilatory response to exercise in the transected animals and only an insignificant portion in the intact animals. This implies: (*1*) that an extra stimulus (or stimuli) operated in both groups; (*2*) that the potency of this extra stimulus was less in the transected group; and (*3*) that the extra stimulus was humorally transmitted in the transected group provided it originated in the exercising limbs. The observed changes in the respiratory responses to exercise following transection of the spinal cord conform to neither of the two limiting predictions based, respectively, on the assumptions of an exclusively neural or an exclusively humoral pathway for the transmission of the exercise stimulus in the intact animal. Under these conditions, no conclusion can be drawn regarding the pathway or pathways actually utilized in the normal animal (Authors' summary).—*Regulation of breathing during electrically induced muscular work in anesthetized dogs following transection of spinal cord, F. S. Grodins & D. P. Morgan, Am. J. Physiol., July 1, 1950, 162: 64.*—(*G. C. Leiner*)

Pulmonary Water Loss.—The evaporation from the lungs of man is about 0.84 mg. of water per ml. of oxygen taken up in the lungs when dry air is inspired. In white rats and mice the evaporation from the total animal was found to be of the same order. This could be anticipated because these animals probably have a minimum of evaporation through the skin, and the lungs account for the larger part of the evaporation. In desert rodents as well as in hamsters and wild mice, the total evaporation was around 0.5 mg. of water per ml. of oxygen utilized. This considerable reduction in pulmonary evaporation is of great importance to the desert rodents in their water balance. The mechanism is not clear, but might be found in a higher utilization of alveolar air or in a reduced water content of the expired air (Authors' summary).—*Pulmonary water loss in desert rodents, B. Schmidt-Nielsen & K. Schmidt-Nielsen, Am. J. Physiol., July 1, 1950, 162: 31.*—(*G. C. Leiner*)

Inspiration and Atrial Inflow.—Analysis of the phasic changes of the effective right atrial pressure provides indirect evidence that the right atrial inflow increases during inspiration. The magnitude of change of the effective atrial pressure gradient during ventricular systole is due to the volume of inflow, since there is no atrial outflow during this period and the volume-elasticity factor is negligible. On the first beat occurring after the onset of inspiration the magnitude of the pressure gradient existing during ventricular systole increases. The increase is not due to changes in pulmonary resistance nor to hydrostatic effect (Authors' summary).—*Further evidence that inspiration increases right atrial inflow, D. F. Opdyke, H. F. van Noate, & G. A. Brecher, Am. J. Physiol., August 1, 1950, 162: 259.*—(*G. C. Leiner*)

PUBLIC HEALTH AND EPIDEMIOLOGY

Relapse Rate in Phthisiologists and Non-phthisiologists.—The relapse rate was compared in 2 groups of physicians who had developed pulmonary tuberculosis as medical students and subsequently recovered. All were treated in the French Student Sanatarium. One group of 141 physicians later became phthisiologists and remained exposed to open cases; the other 122 doctors became general practitioners or specialists in fields other than tuberculosis. Among the phthisiologists were 131 sanatorium physicians, 31 private tuberculosis specialists, and 6 tuberculosis clinic physicians. Students who relapsed less than two years after discharge from the sanatorium were eliminated from this study, as were physicians whose practice as tuberculosis specialists had been shorter than two years. Both groups had comparable social and economic backgrounds. The length of the observation period from the date of discharge was similar, an average of 7.09 years for the phthisiologists and 6.5 years for the non-phthisiologists. The nature of the first episode of active tuberculosis was evaluated according to 5 criteria: (*1*) the initial gravity of the roentgenographic changes; (*2*) the duration of the sanatorium stay; (*3*) the extent of roentgenographic clearing on discharge; (*4*) the presence or absence of prolonged collapse; and (*5*) the time elapsed before the sputum became bacillus-free by the concentration method. A notable difference was found in the 2 groups as far as the roentgenographic lesions were concerned. Benign lesions were found in 44.3 per cent of the future phthisiologists. There were intermediate and grave lesions in 55.7 per cent, whereas only 35.5 per cent of future non-phthisiologists showed intermediate and grave lesions. It is not believed, however, that this difference in the initial roentgenographic picture is relevant for the subsequent relapse rate; the incidence of relapse for the total of cases (phthisiologists and non-phthisiologists) was 20 per cent for benign lesions and 21.8 per cent for intermediate and grave lesions. As far as the other criteria are concerned, no significant differences were noted in the two groups. The relapse rate was 24.8 per cent, or 3.5 relapses per 100 observation years, for the phthisiologists as compared to 11.5 per cent, or 1.75 relapses per 100 observation years, for non-phthisiologists. This double relapse rate in the phthisiologists is statistically significant and is attributed to new exogenous reinfections as a consequence of occupational exposure to tuberculosis. *La reinfection exogene chez le tuberculeux gueri (frequence comparée des rechutes chez les médecins anciens tuberculeux exercant et n'exercant pas la phtisiologie), G. Canetti & M. Robert, Rev. de le tuberc., 1950, 14: 451.*—(*V. Leiles*)

Tuberculosis in Nurses.—At the Adelaide Hospital in Dublin prior to 1920, no precautions were taken to prevent tuberculosis in nurses. Since 1922, roentgenograms have been taken prior to entrance into training. Since 1938, cases of open tuberculosis have been kept off the general wards. Since 1939, Mantoux tests have been done on all probationers. This is repeated every few months and convertors receive roentgenograms. Since 1948, all nurses are roentgenographed every six months, and all Mantoux-negative probationers receive BCG. Between 1920 and 1949 there

was an incidence of tuberculosis in student nurses of 8.6 per cent. Between 1920 and 1938 the incidence was 9.3 per cent. After open cases were removed from the general wards, the incidence fell to 6.8 per cent. During these later years, the incidence was 5.6 per cent in initially Mantoux-positive, and 6.4 per cent in Mantoux-negative, nurses; the difference is not statistically significant. If a nurse converts, she is sent home to rest for three to six months. Many convertors have shown no ill effects.—*Incidence of tuberculosis in nurses in a Dublin hospital and the steps taken to reduce this, G. Bewley, Lancet, March 25, 1950, 1: 586.—(A. G. Cohen)*

Tuberculosis in Prostitutes.—A survey conducted on 146 prostitutes in the Canary Islands revealed that 60 per cent reacted to tuberculin; there were no appreciable variations in the various age groups. The incidence of positive reactors was higher among those coming from metropolitan Spain. Fluoroscopic examination, completed by the usual diagnostic measures in suspicious cases, revealed 6 cases (4.1 per cent) of residual tuberculous lesions and 5 cases (3.4 per cent) of active tuberculosis in need of treatment. Two of the latter had sputum containing tubercle bacilli. Of the surveyed group, 32 had been examined at the dispensary prior to the survey. Among this group, the conversion of the tuberculin test from negative to positive was noted in 4 cases and a previously positive test turned negative in 6 cases. As a by-product of this survey a high incidence of positive serological tests for syphilis was found.—*Encuesta de tuberculosis en prostitutas, T. Cervia & R. Luelmo, Rev. espan. de tuberc., June, 1940, No. 171: 405.—(L. Molnar)*

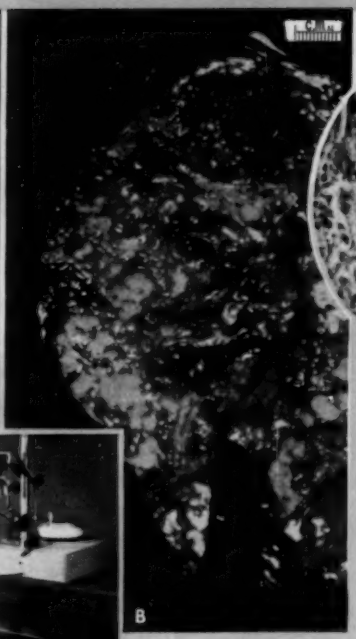
Mass Roentgen Surveys.—Certain policies have to be followed if mass surveys are to be effective in controlling tuberculosis and in uncovering pulmonary neoplasms. Photofluorograms must be overread not underread. The population segment to be studied has to be carefully chosen. Since a significant percentage of persons with symptoms is examined in surveys, the technical staff should be properly trained to distinguish between the true survey case and the person with symptoms by asking some leading questions. The person with symptoms should be referred to a physician or chest clinic. If ex-sanatorium patients report for survey examinations they, too, should be referred for supervision. Patients whose photofluorograms show a grave condition should be recalled immediately for an interview by a chest specialist. Adequate follow-up plans are an integral part of the survey program. An annual roentgenogram of the chest is advisable for all adults, but men over 45 years of age should be surveyed every six months.—*A critical evaluation of mass roentgen surveys, K. R. Boucot & D. A. Cooper, J.A.M.A., April 22, 1950, 142: 1255.—(H. Abeles)*

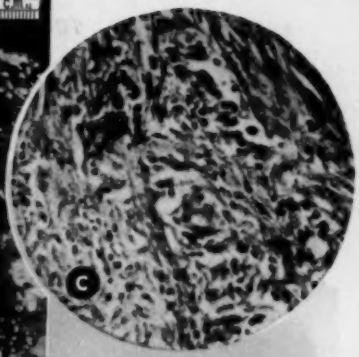
BCG.—Reports on the efficacy of freeze-dried BCG were received from France, Belgian Congo, Phillipines, Malaya, Turkey, and the United States. The material in each case was prepared at the Pasteur Institute in Paris. The results were comparable to those obtained with fresh BCG. Equally good results were obtained by scarification, multiple puncture, or intracutaneous injection. Younger infants were slower in reacting than older children. Allergy caused by BCG always lagged behind that due to fresh BCG. These facts should be born in mind in performing tuberculin tests after vaccination.—*Vaccination with freeze-dried BCG: Preliminary clinical reports, F. van Deinse & F. Sénéchal, Tubercle, August, 1950, 31: 185.—(A. G. Cohen)*

Adenitis from BCG.—A total of 9,577 newborn babies in Uruguay received BCG vaccination in the right deltoid area by the Rosenthal-Etcheverry technique of 3 skin punctures through each of 5 drops of vaccine. The babies were closely watched. Regional axillary adenopathy developed in 129 cases (1.34 per cent). These adenopathies usually became apparent two or three months after the vaccination. As a rule they were slow-growing and some became as large as a hen's egg. Supraclavicular adenopathy was

rare. The glands were usually single but some were multiple. There was no periadenitis producing adhesions to adjacent structures or to the skin, the glands were not painful, and they exerted no untoward effect on the general well-being of the baby, its development, or growth. In all the children with adenitis there was considerable scarring at the site of inoculation. This suggests a connection between too deep scarifying and the development of subsequent adenitis as a result of overdosage of bacilli. In these children it was also noted that the allergic responses obtained had a higher intensity than in those who did not develop glandular reactions. The natural history of the adenopathies was variable. Some cases regressed completely. Others required aspiration, and still others ruptured spontaneously. In this series, 75 (58.14 per cent) suppurated. Of these, 38 cases were studied bacteriologically. In 24 (63.4 per cent) acid-fast bacilli were found; in 6 (15.8 per cent) pyogenic organisms were found; in 8 cases (21.06 per cent) the studies were negative. In view of the fact that some authors have described mediastinal glandular involvement with BCG vaccination, all of the children in this series had roentgenograms of the thorax. In all the cases these were negative.—*Complicaciones ganglionares en los calmettizados, C. Epifanio & R. Gorlero Bacigalupi, Hoja Tisiol., March, 1950, 10: 1.*—(*F. Pérez-Pina*)

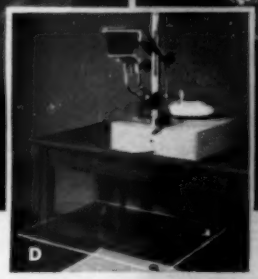
Lung Changes in Corundum Smelters.—In the latter years of the last war, 25 cases of pulmonary disease were reported in Western Germany in an industrial plant producing corundum, known also as carborundum, an artificial abrasive containing aluminum obtained from bauxite through heating in electric furnaces. The reported cases were all observed in individuals directly exposed to the fumes evolved during the melting process. Fifteen of these cases were recognized as pulmonary fibrosis of industrial origin; 10 of the patients died. Many of them had worked six to twenty-two years in the same plant. The fact that pulmonary lesions appeared only in 1942 may be explained by war-time working conditions such as the black-outs, the larger number of furnaces producing the abrasive in the same space, and the long working hours. The patients complained mostly of cough and dyspnea; spontaneous pneumothorax was noted in 3 cases. The clinical course was a rapid one; in some of the cases death occurred a few months after the appearance of the first symptoms. Physical examination showed only signs of bronchitis but the vital capacity was reduced in most cases to 1,000 cc. In 5 cases active tuberculosis was associated with the syndrome. Radiologically all the patients had enlargement and thickening of the hilar shadows, increase of the bronchovascular markings, nodular densities, or larger area of opacity. Irregularities of the mediastinal shadow seemed to be characteristic. The clinical picture and the radiologic findings were suggestive of the known syndrome caused by aluminum inhalation. Post-mortem examination of 7 of the deceased patients showed the histologic features characteristic of "aluminum lung." The lungs appeared shrunken, the cut surfaces were slate-gray, and there was marked fibrosis. Histologically, fibrous thickening of the alveolar septa, hyaline changes in the alveolar walls, and obliteration of many alveoli were noted. Chemical analysis of lungs of 2 deceased individuals showed a markedly increased aluminum content. In "aluminum lung," the changes are produced by the inhalation of metallic aluminum. The aspirated aluminum dust is ionized in the pulmonary tissue fluids and aluminum ion precipitates the tissue proteins producing the histologic changes. It is assumed that the fumes liberated in the process of melting bauxite contain some product that acts similarly. It is proposed that work in this industry should be classified as a professional hazard, and diseases developed therefrom should be compensable.—*Ueber Lungenveraenderungen bei Korundschmelzern, J. Hagen, Deutsche med. Wchnschr., March 24, 1950, No. 12: 399.*—(*L. Molnar*)





Reproductions A *and* B *show gross aspects of diseased lung after pneumonectomy. Reproduction* C *shows photomicrograph made from histological section of affected lung.*

Illustration D *shows setup of Kodak Flurolite Enlarger fitted with Camera components for the photography of gross specimens.*



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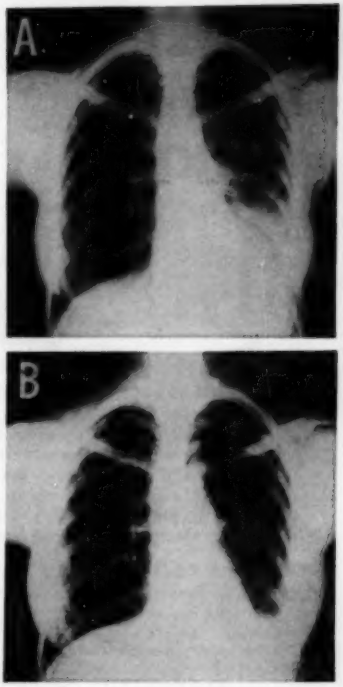

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Tablets, 0.5 Gm., 0.25 Gm.; *Capsules*, 0.25 Gm.; *Elixir*, 0.1 Gm. per cc.; *Solution*, 2% (intravenous).

Tolserol *(Squibb Mephenesin)*

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MANUFACTURING CHEMISTS TO THE MEDICAL PROFESSION SINCE 1858

"TOLSEROL" IS A REGISTERED TRADEMARK OF E. R. SQUIBB & SONS

EFFERVESCENT PARASAL* TABLETS

... the logical solution to the many problems of P. A. S. administration

In the P.A.S. therapy of tuberculosis, EFFERVESCENT PARASAL TABLETS —newest of Panray's formulations— are fast becoming the preferred dosage form for children and adults. One tablet dropped into water effervesces and dissolves almost instantly, providing 2 grams of active P.A.S. in a pleasantly-flavored, clear solution.

GREATER ACCEPTABILITY to the PATIENT: EFFERVESCENT PARASAL TABLETS are pleasantly mint flavored. They can be taken in water, milk, or fruit juices. And the patient need no longer swallow a huge number of tablets every day. Just 1½ tablets q.i.d. is sufficient to give the average dose of 12 gms. daily.

GREATER CONVENIENCE: Now you need no longer worry about time-consuming filtrations, storage problems, decomposition of solutions, etc. EFFERVESCENT PARASAL TABLETS, bisected for even greater convenience, *always and instantly provide a fresh, fully potent solution.*

GREATER ECONOMY: EFFERVESCENT PARASAL TABLETS eliminate the high costs of extra labor . . . wasted batches

. . . refrigeration . . . time-consuming calculations. You simply use tablets as needed. Yet the equivalent cost is no higher than that of other Parasal dosage forms.

MORE ACTIVITY PER TABLET: EFFERVESCENT PARASAL TABLETS have the highest potency of any P.A.S. tablet yet produced. Each tablet contains 2 grams of active Parasal, *4 times as much as any Parasal tablet designed for oral ingestion.*

MORE RAPID ABSORPTION: Since the patient ingests a solution rather than tablets or granules, it is almost certain that the drug will be rapidly and completely absorbed. *Samples and prices on request.*

*Panray's brand of Para Aminosalicylic Acid

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A SPECIAL ANNOUNCEMENT TO SUBSCRIBERS

For more than five years, the American Review of Tuberculosis has been waging a losing battle against advancing production costs. The prices of paper, type-setting, presswork, and binding have steadily mounted, and the end is not yet in sight.

With reluctance, therefore, we are forced to announce an increase in the subscription rate from $3.00 to $4.00, *effective January 1950.*

THE AMERICAN REVIEW OF TUBERCULOSIS
1790 Broadway • New York, N. Y.

NATIONAL TUBERCULOSIS ASSOCIATION

Annual Meeting **May 15–18, 1951**

Cincinnati, Ohio

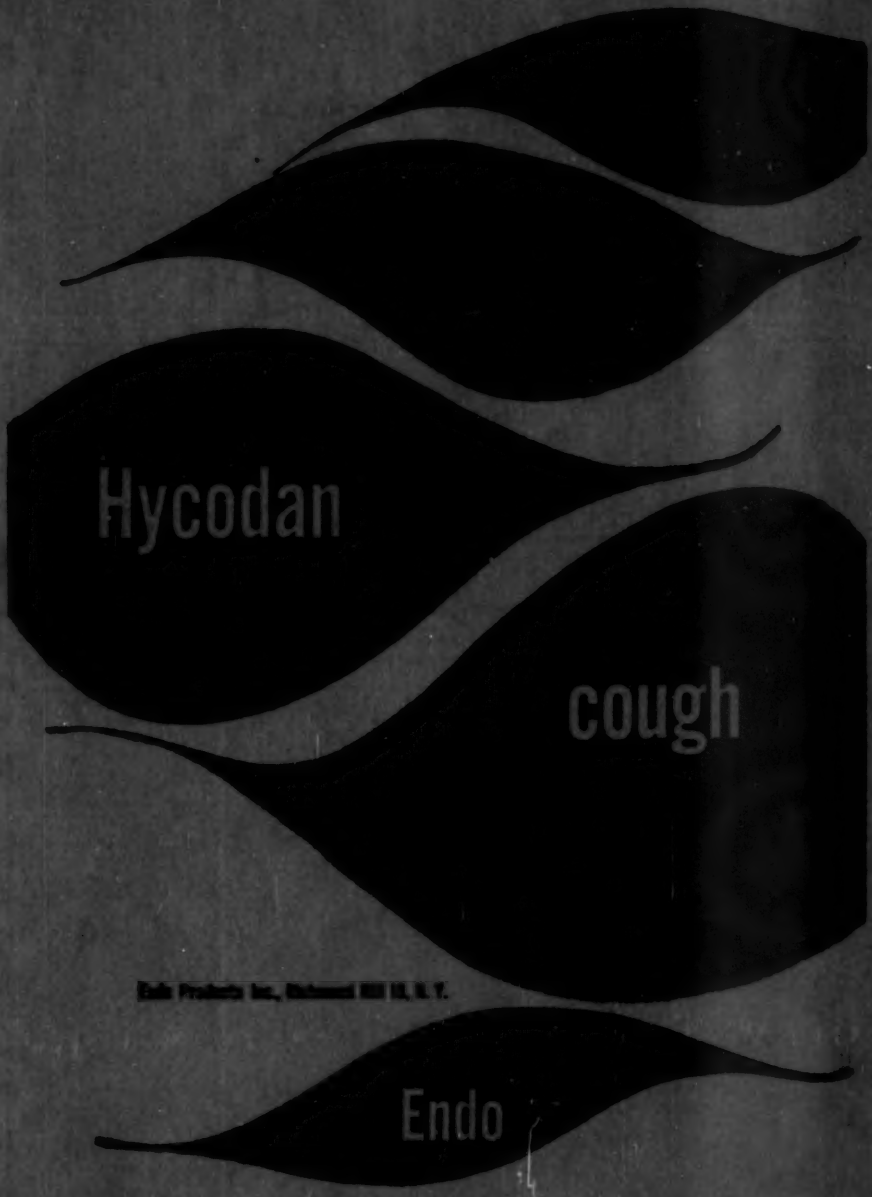
The annual meeting of the National Tuberculosis Association will be held in Cincinnati, Ohio, May 15-18, 1951. The Netherland Plaza Hotel will be headquarters.

Some 1500 rooms have been promised by the hotels listed below for the accommodation of those attending the meeting. Arrange to share a double room if possible.

Application for room reservations should be made directly with the hotel of your choice.

Hotel	*Address*	*Single*	*Double*
Netherland Plaza	Carew Tower	$4.00–$10.00	$9.00–$13.00
Sheraton-Gibson	421 Walnut Street	$4.00–$12.00	$7.50–$12.00
Terrace Plaza	15 West Sixth Street	$7.00–$12.00	$10.00–$15.00
Sutton Hotel	4th at Vine Street	$4.00–$10.00	$8.00–$12.00
Fountain Square	420 Vine Street	$4.00–$5.50	$8.00–$8.50
Palace Hotel	6th at Vine Street	$2.50–$4.00	$5.00–$7.00
Metropole Hotel	[illegible]9 Walnut Street	$3.00–$6.00	$6.50–$10.00
Broadway Hotel	[illegible] and Broadway	$3.50	$5.50–$6.50
Alms Hotel	Victory Pkwy. & McMillan	————	$7.50–$15.00

See February issue of NTA Bulletin for preliminary program.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